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Postirradiation Changes in the Levels of Organic Phosphorus in the Blood of Patients with Leukemia*†

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INTRODUCTION

Tracer doses of radioactive phosphorus, P^{32}, have been used by Lawrence and co-workers (4) to study the metabolism of phosphorus in leukemic mice. Lawrence was interested primarily in the distribution of P^{32} among the various groups of phosphorus compounds in the animal tissues. It seemed desirable, however, to extend his experiments to the leukemic patient and to measure, after the administration of small quantities of P^{32}, the concentration of both radioactive and nonradioactive phosphorus in the various phosphorus-containing fractions of the patient's blood cells. These fractions included the inorganic and organic acid-soluble (O.A.S.P.) compounds of the erythrocytes, of the leukocytes, and of whole blood.

It was noted that the ingestion of a subtherapeutic dose of approximately 1.5 mc. of radioactive phosphorus, P^{32}, by the first of the patients investigated was followed by a rapid increase in the concentration of the organic acid-soluble phosphorus compounds of the erythrocytes. Subsequently, an increased concentration in both erythrocytes and leukocytes as a sequel to the administration of P^{32} was observed in 4 other leukemic patients, but it has not followed the administration of nonradioactive phosphorus to the same individuals.

It was desirable to establish whether the observed change in the organic acid-soluble phosphorus levels of the blood cells was due to the radioactivity of the P^{32} or to some chemical property of this isotope. If to the former, similar changes would be expected after the administration of comparable doses of x-rays. Accordingly, such doses were administered to several patients by two methods of external irradiation.

The first method used was whole body irradiation.[1] This was chosen because it distributes the radiation throughout the entire body, a distribution comparable to that effected by the administration of P^{32}. The second method was the direct exposure of the cardiac area, and was employed to ascertain the change in organic acid-soluble phosphorus levels which followed irradiation essentially limited to the circulating blood.

METHODS

The concentrations of inorganic phosphorus and of organic acid-soluble phosphorus compounds of whole blood, erythrocytes, and leukocytes were measured by the method of Fiske and Subbarow (2).

The erythrocytes and the leukocytes were readily separated from the whole blood of leukemic patients. The cells from 20 cc. of whole oxalated blood were allowed to settle for 15 minutes. The sedimentation rate of the red cells of leukemic blood is much more rapid than that of the leukocytes, which thus remained suspended in the supernatant plasma. The plasma, with its contained leukocytes, was pipetted carefully into a centrifuge tube, and then centrifuged at 3,000 r.p.m. for 15 minutes. The supernatant cleared plasma was removed as completely as possible, and the packed white cells resuspended in 5 cc. of isotonic saline. The concentration of white cells in this suspension was determined by its hematocrit value. Similarly, a sus-

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[1] This was administered in the Heublein (1) unit of the Memorial Hospital. In this communication, "Heublein" is synonymous with "whole body radiation."

pension of known concentration was made from the sedimented erythrocytes.

To test the reliability of the technic used to separate the red and white cells, the inorganic and total acid-soluble phosphorus concentrations were determined in samples of whole blood and in the erythrocytes, leukocytes, and plasma of those same samples. Four such experiments were done, and the sum of the amounts of phosphorus found in the erythrocytes, leukocytes, and plasma in each of the four equalled the amount found in an equivalent amount of whole blood.

Phosphorus determinations were made daily while the patients were fasting, and also 3 hours after their evening meal.

The nonradioactive phosphorus used in this investigation was an aqueous solution of Na_2HPO_4, containing from 15 to 30 mgm. per cc. This was administered to each of the 6 patients studied.

The radioactive phosphorus used was an aqueous solution of Na_2HPO_4 which contained from 15 to 30 mgm. per cc. and had an activity of from 100 to 200 μc. per cc.

The whole body radiation was administered by a 185 kv. machine at a distance of 500 cm. and at a rate of 0.4 r per hour.

The cardiac radiation was administered by a 150 kv. machine with 1.0 mm. Cu. filter, at a distance of 60 cm. through a 10 cm. cone. The rate of output was from 2 to 4 r per min. Under these conditions, it has been calculated that the delivery of 20 r (air) to the precordial area would administer an effective dose of not more than 2 r to the entire volume of blood as it circulated through the heart during the irradiation period.

Clinical Material

The clinical material comprised 6 patients with chronic leukemia. These were all adults; 5 males and 1 female.

Of the 6 patients, 4 had myelogenous and 2 lymphatic leukemia. One of the latter was in an aleukemic phase of his disease. The diagnosis in each case was established by the usual criteria of physical findings and hematologic picture. Marrow aspiration biopsies confirmed the diagnosis in all instances.

All of the 6 patients remained afebrile during the course of this investigation. In no case was any significant hematologic change noted.

Four (G.V., S.G., J.L.P., P.S.) of the 6 patients were on a constant diet of 180 gm. of carbohydrate, 100 gm. of fat and 80 gm. of protein. This diet was not modified during the period of observation. Before any form of radiation was administered to these 4 patients, they had taken a standard diet for from 10 to 15 days in order that the concentration of the organic acid-soluble phosphorus compounds in the blood cells might reach a stable level.

Results

The results obtained, while qualitatively uniform, varied quantitatively from case to case. Hence, it is impossible to draw a curve to show the average effect of the ingestion of P^{32} or of the radiation on the intracellular organic phosphorus. It is necessary, therefore, to consider separately the results found in each patient.

G.V. This patient had been on a standard diet for 10 days when he was given nonradioactive phosphorus. For the 5 days previous to the administration, the organic acid-soluble phosphorus (O.A.S.P.) concentration of his blood cells ranged from 60 to 88 mgm. per 100 cc. of leukocytes, and from 46 to 62 mgm. per 100 cc. of erythrocytes (Fig. 1).

No rise in the O.A.S.P. levels followed the administration of 450 mgm. of nonradioactive phosphorus. For the next 4 days the O.A.S.P. levels of the leukocytes ranged from 65 to 88 mgm. per 100 cc. of cells, and of the erythrocytes from 55 to 60 mgm. per 100 cc. of cells.

The oral administration of 1.4 mc. of P^{32} in a total of 450 mgm. of Na_2HPO_4 was followed by a rise of the O.A.S.P. in the leukocytes from 82 mgm. per 100 cc. to 146 mgm. per 100 cc. in 60 hours. During the next 13 days the value gradually returned to its original level. No significant change in the concentration of the O.A.S.P. fraction was noted in the erythrocytes during this period.

At this time, 2 weeks after the administration of P^{32}, the patient was exposed to 3.1 r of whole body x-radiation. Within 3 hours the O.A.S.P. of the leukocytes rose from 68 mgm. per 100 cc. to 80 mgm. per 100 cc., reached a peak value of 220 mgm. per 100 cc. in 4 days, and did not return to its original level until 12 days later. The O.A.S.P. of the erythrocytes also rose within 3 hours from a level of 44 mgm. per 100 cc. to 56 mgm. per 100 cc., reached a peak of 94 mgm. per 100 cc. within 4 days, and remained about 65 to 75 mgm. per 100 cc. even after 17 days.

Seventeen days after the whole body irradiation, the blood of this patient was irradiated, 20 r of x-ray being administered to a field over the heart. The exposure was of 10 minutes' duration, and was followed within 2 hours by a sharp rise in the O.A.S.P. of the leukocytes from 72 to 125 mgm. per 100 cc., but by a fall from 75 to 42 mgm. per 100 cc. in the red blood cells. During the next 11 days, the concentrations of the O.A.S.P. fraction in both white cells and red cells fluctuated over wide ranges from day to day (Fig. 2).

S.G. During the last 4 of 7 days on which this patient was taking the standard diet, the blood cell O.A.S.P. ranged from 60 to 89 mgm. per 100 cc. of leukocytes, and from 52 to 72 mgm. per 100 cc. of erythrocytes. The values obtained during the 7 days after the oral administration of 450 mgm. of nonradioactive Na_2HPO_4 ranged from 52 to 78 mgm. per 100 cc. of white blood cells, and from 40 to 72 mgm. per 100 cc. of red blood cells (Fig. 3).

X-ray was first administered to this patient in the form of local cardiac irradiation; 20 r were delivered in 5.5 minutes. In both the erythrocytes and leukocytes a rise in the O.A.S.P. resulted. That of the leukocytes rose from 52 to 120 mgm. per 100 cc. and returned to its original level within 48 hours; that of the erythrocytes rose from 62 to 108 mgm. per 100 cc.,

but then fell to the low level of 30 mgm. per 100 cc. within the succeeding 24 hours.

Ten days after cardiac irradiation was administered, the patient was fed 1.4 mc. of P^{32}. Within 12 hours after the ingestion, the O.A.S.P. of the leukocytes rose from 60 to 82 mgm per 100 cc. This rise was succeeded by a prompt fall to 28 mgm. per 100 cc. in the next 12 hours. The O.A.S.P. fraction of the Rbc. rose from 62 to 82 mgm. per 100 cc. within 12 hours.

Twelve days subsequent to the administration of P^{32}, this patient was subjected to whole body irradiation. Five r were delivered in 18 consecutive hours on each of 3 succeeding days; a total of 15 r thus were delivered over a 72-hour period. This irradiation was followed each day by a rise in the O.A.S.P. fraction from 70 to about 96 mgm. per 100 cc. in the leukocytes, and from 50 to about 90 mgm. per 100 cc. in the erythrocytes. In both cells the peak of the rise occurred during the administration of the x-radiation.

It should be noted that in this case the changes of O.A.S.P. were observed shortly after the administration of x-ray, and that the O.A.S.P. levels after radioactive phosphorus persisted for about 72 hours (Fig. 3).

J.L.P. This patient had been on the standard diet for 15 days before he was given nonradioactive phosphorus. During the last 5 days of the control period, the organic acid-soluble phosphorus (O.A.S.P.) concentration of his blood cells ranged from 72 to 92 mgm. per 100 cc. of leukocytes, and from 52 to 62 mgm. per 100 cc. of erythrocytes (Fig. 4).

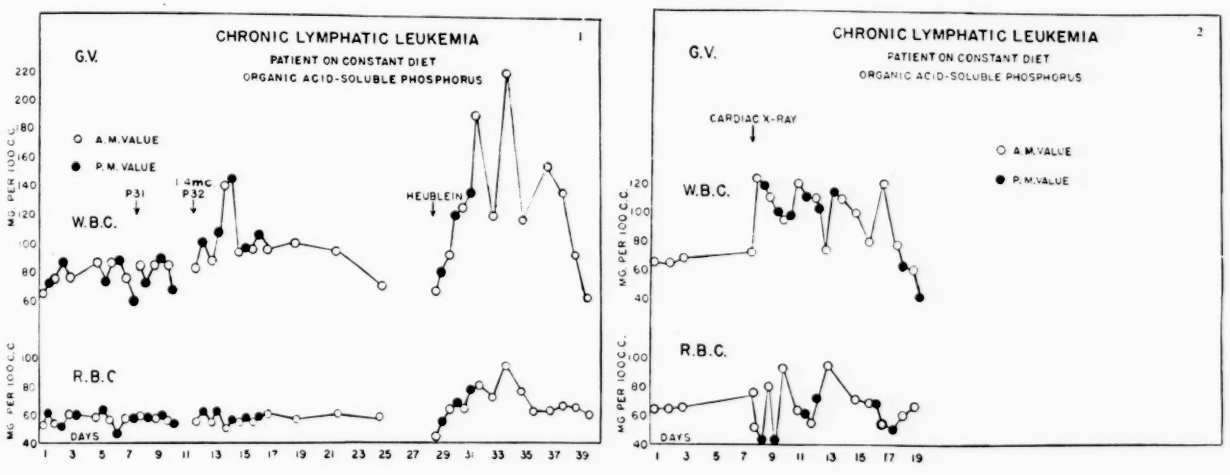
The organic acid-soluble phosphorus (O.A.S.P.) levels of the leukocytes and erythrocytes did not change during the 3 days following the oral administration of 450 mgm. of nonradioactive Na_2HPO_4. However, 48 hours after the oral administration of 1.4 mc. of P^{32} in a total of 450 mgm. of Na_2HPO_4, the O.A.S.P. rose in the white cells from 63 to 114 mgm. per 100 cc., and in the red cells, from 50 to 70 mgm. per 100 cc.

Ten days after the administration of P^{32}, the patient was exposed to 3.0 r whole body x-radiation. This was followed by an increase in the concentration of both red and white blood cell O.A.S.P. The increase in concentration first was noted 6 hours after the exposure, and reached its maximum in from 48 to 72 hours, when the white cell O.A.S.P. had risen from 50 to 110 mgm. per 100 cc., and the red cell O.A.S.P. from 40 to 86 mgm. per 100 cc.

P.S. Cardiac x-ray was the only type of radiation administered to this patient.

Previous to this irradiation, the O.A.S.P. of his white cells ranged from 66 to 76 mgm. per 100 cc., and that of his red cells from 58 to 82 mgm. per 100 cc. (Fig. 5).

After 20 r of x-ray were delivered over the precordium through a 10 cm. cone in 5.5 minutes, the O.A.S.P. of the leukocytes rose from 82 to 136 mgm. per 100 cc. within 24 hours, and that of the erythrocytes from 57 to 86 mgm. per 100 cc. within 48 hours. The O.A.S.P. concentrations of both red and white cells returned to their base levels within 24 hours after the maximum effects were noted.



FIGS. 1 AND 2

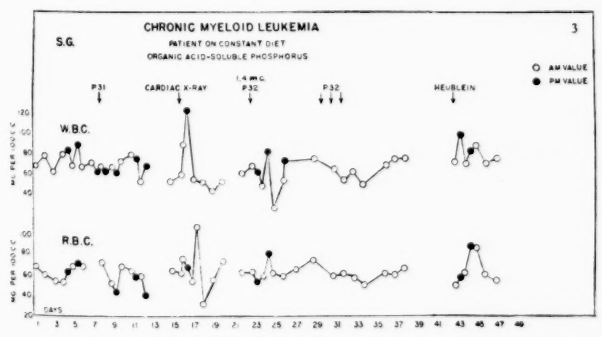


FIG. 3

C.K. This patient was the first used in this study. He had not been on a controlled diet, and was given radioactive phosphorus before the nonradioactive material. The O.A.S.P. concentration of only the red cells could be determined because this patient was in an aleukemic state and not enough leukocytes could be collected to study.

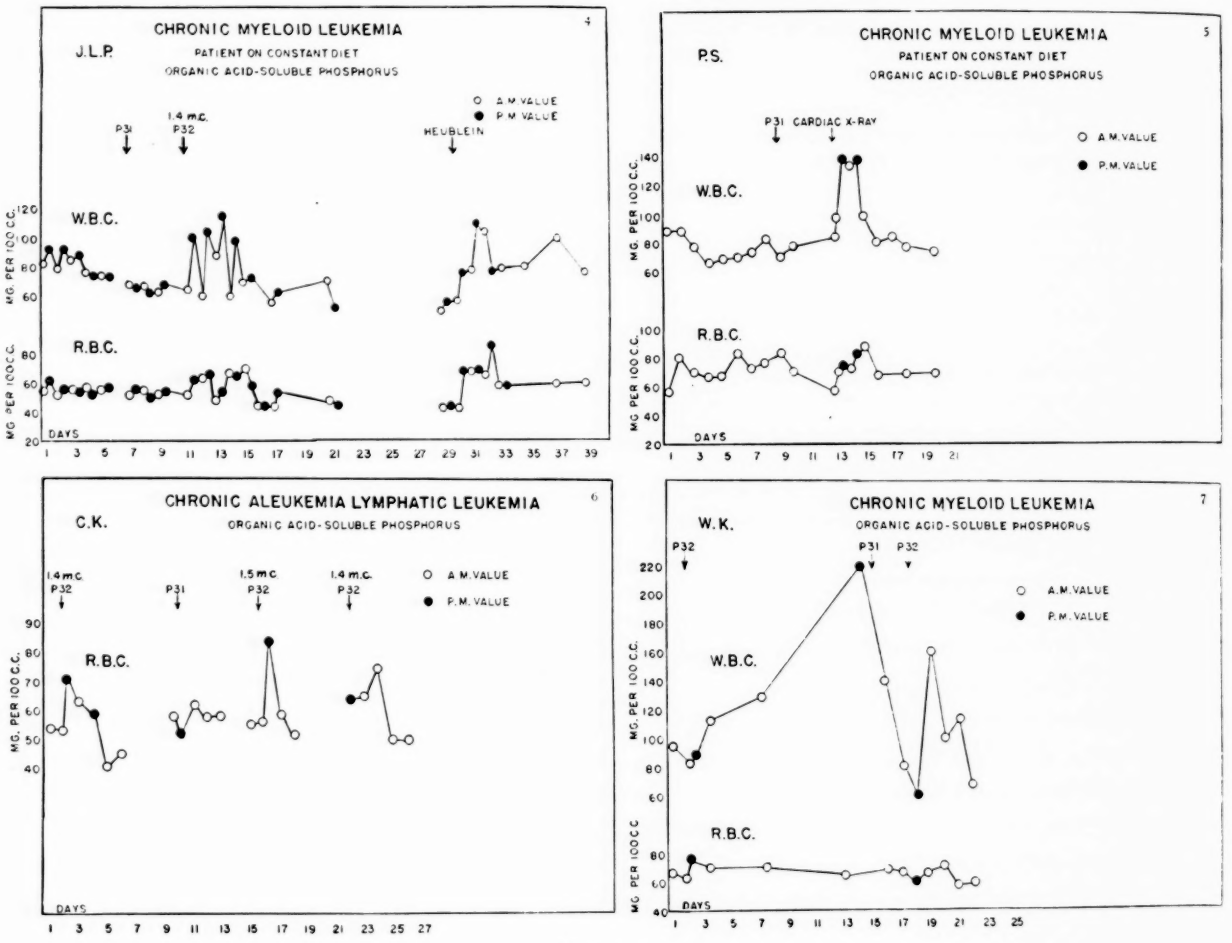
After the first dose of 1.4 mc. of P^{32}, the O.A.S.P. of the erythrocytes rose within 12 hours from 54 to 70 mgm. per 100 cc. The administration of second and third doses of 1.5 mc. each of P^{32} was followed by a rise in O.A.S.P. of the erythrocytes from 55 to 84 mgm., and from 65 to 75 mgm. per 100 cc., respectively (Fig. 6).

No rise in the O.A.S.P. of the red cells was obtained after the ingestion of nonradioactive phosphorus.

The interesting finding to be noted in this patient is that a rise in the O.A.S.P. in the erythrocytes could be effected after each of 3 doses of P^{32}.

W.K. Like the preceeding case, this patient was not on a constant diet when studied. The ingestion of 1.5 mc. of radioactive phosphorus in a total of 450 mgm. of Na_2HPO_4 was

followed by a rise in the O.A.S.P. of his leukocytes from 90 to 220 mgm. per 100 cc. The maximum rise was reached in 12 days. At the time when the maximum leukocyte O.A.S.P. concentration was noted, 450 mgm. of nonradioactive Na_2HPO_4 was administered to the patient, but no further rise in the leukocyte concentration of O.A.S.P. was observed (Fig. 7).



FIGS. 4 TO 7

A second rise in the cell O.A.S.P. fraction was effected after another dose of 1.98 mgm. of P^{32} in 450 mgm. of Na_2HPO_4. In this instance, the level rose from 60 to 166 mgm. per 100 cc. within 24 hours.

Throughout the study in this patient, no significant change was noted in the erythrocyte level of O.A.S.P.

DISCUSSION

This investigation has been confined to a single organ in a single form of malignant neoplastic disease —the blood in leukemia. The group of patients studied was small and the results obtained have varied quantitatively from patient to patient. The one characteristic, however, which binds the group together is that in all patients there was a definite alteration of the organic acid-soluble phosphorus levels in the leukocytes, and usually of the erythrocytes, after all types of radiation.

The technical difficulties in the measurement of the phosphorus content of the small amounts of leukocytes which could be obtained from permissible samples of normal blood have, for the present, prevented the study of a control group of normal subjects. Likewise, the phosphorus metabolism of other organs or tissues subjected to irradiation as yet has not been studied. Therefore, it must be remembered that the observed changes may represent a radiation effect seen only in the blood of patients with leukemia.

As a rule, the evening values of organic acid-soluble phosphorus measured during both the control period and after the ingestion of nonradioactive phosphorus were somewhat higher than were the fasting levels. This was probably due to the ingestion of food. However, after the administration of any form of radiation, the variations between the fasting and nonfasting levels were markedly accentuated. The presentation, both of the fasting and nonfasting levels, was not done under the impression that they are truly comparable values, but to show the increase in the variation between these levels after radiation.

Evidence has been presented to show that the alterations of the organic acid-soluble phosphorus of the

blood cells following the administration of P^{32} are probably due to the radioactivity of the isotope, since no similar alteration followed the ingestion of nonradioactive phosphorus. Furthermore, external x-irradiation produced alterations similar to those which resulted from the administration of the radioactive isotope.

At the present time, a great number of investigations on metabolism are being conducted with various radioactive elements. It is quite possible that the results of some of these studies may not represent normal physiology, but rather the metabolism existing during irradiation. It is not suggested that all metabolic experiments conducted with radioactive isotopes are at fault because radiation has affected the results, but the possibility that some of them have been affected by the radiation must be considered. This possibility warrants particular attention in those investigations in which radioactive phosphorus was used to study phosphorus metabolism.

The objection may be advanced that larger than tracer doses of the isotope were used in this investigation. However, from 1 to 5 μc. in a 30 gm. mouse is a frequent tracer dose. This amount may be considered equivalent to from 2 to 10 mc. in a 60 kg. adult human being. In each instance, the amount of radioactive phosphorus administered to the patients studied was less than 1.5 mc. It is believed, therefore, that while the amount of radioactive isotope used is more than is necessary for tracer work with humans, it is quite comparable to the amount used in animals.

This fact is brought out more clearly by the calculation that if 1 mc. of radioactive phosphorus is retained by a 70 kg. adult for 24 hours, 0.6 "equivalent roentgens" (3) of the whole body radiation is administered. In the case of a 30 gm. mouse, it is found that retention of 1 μc. for 24 hours will administer to that mouse about 1.3 "equivalent roentgens" of whole body radiation. Hence, 5 μc., a not uncommon tracer dose, would, in 24 hours, deliver 6.5 "equivalent roentgens" to the animal. This is almost twice the whole body dose of radiation administered to the patients in the present investigation, which has been shown to cause the marked effects on the phosphorus metabolism of the blood cells. Obviously, then, the radiation accompanying a 5 μc. dose of P^{32} to a mouse, or any comparable amount of the isotope to other animals, cannot be regarded as negligible in metabolism studies.

In all likelihood, the alterations of total acid-soluble phosphorus levels in leukemic leukocytes and erythrocytes are not the primary effect of the irradiation. Probably these alterations are only an indication of a disturbance of one or more of those systems which control phosphorylation. It is those factors which must be studied carefully before any interpretation of the radiation effect on phosphorus metabolism is possible.

The organic acid-soluble phosphorus compounds are connected intimately with the cellular enzymes which control respiration and carbohydrate metabolism. The alteration of the organic acid-soluble phosphorus values suggests that the radiation has in some degree affected these enzyme systems. Such a disturbance of these systems might prove detrimental to the normal functioning of the cell. It is possible, therefore, that when therapeutic amounts of radiation are administered, these enzyme systems are so profoundly disturbed that no recovery to the normal state is possible, and, as a result, the cell is permanently damaged or killed.

It is the opinion of the authors that the biochemical effects after very small doses of irradiation, as noted in this paper, are the first of that nature to be observed. It would appear that the alterations of phosphorus metabolism are, at present, the most sensitive index of exposure to radiation.

Summary and Conclusions

1. The administration of subtherapeutic amounts of radioactive phosphorus to 5 patients with leukemia has been followed by an alteration of the organic acid-soluble phosphorus fraction of their blood cells.

2. The administration of nonradioactive phosphorus to 6 patients never was followed by any significant alteration of the organic acid-soluble phosphorus of the blood cells.

3. These same alterations were observed after the administration of very small doses of whole body x-irradiation to 3 patients, and after irradiation of the blood through a precordial port to 3 patients.

4. The amount of radiation delivered by the tracer doses of radioactive isotopes used in metabolism studies cannot be regarded as negligible. It is possible that some of these studies have measured the metabolic changes consequent upon the radiation.

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The Isotopic Constitution of Potassium in Animal Tumors and Muscle from Tumor-Bearing Animals

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Potassium is known to play an important role in the vital processes of both normal and tumor cells. Since it is possible that the mechanism of its function in the tumor cell is somewhat different from that in the normal cell, it was thought that a comparative study of the isotopic constitution of potassium as contained in normal and tumor tissue might contribute towards the elucidation of such a difference. In the present paper results are described of investigations continuing earlier studies (3) on potassium in normal and tumor tissue from animals. The investigations were concerned with the two main isotopes, K^{39} and K^{41}, since the third isotope, K^{40}, exists only in very minute quantity. In addition, comparison was made of the potassium in muscle tissue from normal and tumor-bearing animals.

Materials and Methods

Preparation of ash.—The neoplasms used were Jensen rat sarcoma and mouse sarcoma S 37. Tumors were produced by subcutaneous inoculation of minced tumor tissue in rats of various ages and in adult mice. The animals were chloroformed 13 to 20 days after inoculation. After being freed as far as possible from adhering connective tissue, etc., the whole of each tumor consisting of the living area and the necrotic, frequently hemorrhagic, portion, was cut into a number of small pieces; or small pieces of tissue were taken from the living area only. The pieces were rapidly rinsed in distilled water and dried on Petri dishes at about 100° C. The dry material obtained from tumors of the same batch was mixed and then ashed, wholly or in part. The ashing was done in a platinum dish which had been heated to bright redness to remove any potassium absorbed by the metal. Ashes from a series of normal tissues (including red blood corpuscles and blood plasma) were prepared in a similar way. The tissues were taken from normal rats, and, in addition, muscle was taken from both normal and tumor-bearing rats and mice. By bleeding the animal the blood content of the solid tissues was lowered appreciably.

Mass spectrographic measurement.—The ash was used, without further treatment, for the determination of the isotopic ratio K^{39}/K^{41} by means of a mass spectrograph. Detailed descriptions of the apparatus and procedure were given in previous communications (1, 4). From the isotopic ratio the percentage of the heavy isotope K^{41} was calculated (neglecting K^{40}). About thirty estimations were made in succession on each sample of ash; the average of any ten estimations usually agreed to within ± 0.01, corresponding to ~ 0.005 per cent K^{41}.

Results

Ordinary commercial potassium chloride (A.R.) was utilized as a standard in respect of mineral potassium. For the mass spectrographic analysis the chloride was converted into the perchlorate. The isotopic ratio determined with this preparation was 14.20, and the K^{41} content was accordingly 6.579 per cent.

The results with ash from tumor tissue are shown in Table I. It will be seen that the isotopic ratio was distinctly higher than that of mineral potassium, hence the content of K^{41} was correspondingly lower. The deviation was observed in samples from both Jensen rat sarcoma and mouse sarcoma S 37. As regards Jensen sarcoma, the age of the animal apparently had little influence, and similar values were found with ashes from the whole of the tumor and from the living area alone. The increase in the content of K^{41} varied between 0.9 and 1.3 per cent, the average being 1.1 per cent. It is to be noted that a corresponding result was obtained with a spontaneous rat tumor situated in the subcutaneous tissue. Microscopic examination showed a fibroma-like structure with a relatively small number of cells and large number of collagen fibrils. The isotopic ratio was 14.38, and the K^{41} content accordingly 6.502 per cent.

A study was made of the isotopic constitution of potassium in various normal tissues, and a detailed account of the results was given in a previous paper (4). The following solid tissues were investigated: liver, kidney, lung, salivary gland, skeletal muscle,

heart muscle, brain, spleen, lymph glands, testis, and bone including marrow from adult and preadult rats, liver, kidney, and skeletal muscle from newborn rats. It was found that the isotopic ratio of potassium in the ashes of these tissues was usually very close to that of mineral potassium. In a few exceptional cases the ratio was increased slightly, and the K^{41} content accordingly decreased (by 0.4 to 0.7 per cent). The only tissue which showed a marked and regular deviation was bone, including marrow; here the isotopic ratio varied in 5 samples between 13.79 and 13.97, corresponding to 6.761 and 6.680 per cent K^{41}. Compared to mineral potassium, the content of K^{41} was increased by 1.5 to 2.8 per cent, the average being 1.9 per cent. A similar deviation was obtained with potassium present in the blood plasma of rats; in 5 samples the isotopic ratio showed values between 13.72 and 13.92, corresponding to 6.793 and 6.702 per cent K^{41}. The increase in K^{41} content was therefore 1.8 to 3.2 per cent, the average being 2.5 per cent. On the other hand, potassium contained in red blood corpuscles showed a normal isotopic ratio.

It will be of interest to compare the isotopic constitution of potassium in corresponding tissues from normal and tumor-bearing animals. This has been done so far with skeletal muscle, taken mainly from fore- and hind-legs. Table II shows the results with muscle from normal rats and mice. It is evident that the isotopic ratio was generally the same as that of mineral potassium. Only one sample from rat muscle (No. 1, Table II) showed a minute increase, and a corresponding decrease in the K^{41} content (by 0.4 per cent). A normal isotopic ratio was also obtained with muscle tissue from newborn rats. The results with muscle from tumor-bearing rats and mice are given in Table III. It will be seen that the isotopic ratio showed a distinct increase in each case. The corresponding decrease in the content of K^{41} was, on an average, about 1.0 per cent, thus of similar magnitude as that obtained with tumor tissue. Sample No. 3, Table II, and sample No. 3, Table III, are of special interest, each being prepared from the muscle tissue of 2 female rats. The 4 animals, kept under identical conditions and of similar weight, were inoculated with the same amount of minced sarcoma tissue. Two animals developed tumors, while the two others were resistant, and therefore considered as normal. The muscle from the tumor-bearing animals constituted sample No. 3,

TABLE I: ANALYSIS OF POTASSIUM IN JENSEN RAT SARCOMA AND MOUSE SARCOMA S 37

Sample no.	Tumor from	No. of animals (and tumors) used	Average animal weight (without tumor) in gm. (approx.)	Average tumor weight in gm. (approx.)	Days after inoculation	Isotopic ratio K^{39}/K^{41}	Per cent of K^{41}
1.	Rat	5	235	15.5	18	14.40	6.494
2.	Rat	14	80	14.0	14	14.35	6.515
3.*	Rat	10	80	14.5	14	14.38	6.502
4.*	Rat	8	160	13.5	13	14.33	6.523
5.	Mouse	6	30	2.0	20	14.38	6.502
6.	Mouse	2	35	2.0	13	14.36	6.510

* Samples Nos. 3 and 4 were prepared from the living areas only, the other samples from the whole tumors.

TABLE II: ANALYSIS OF POTASSIUM IN SKELETAL MUSCLE FROM NORMAL RATS AND MICE

Sample no.	Species	No. of animals used	Average animal weight in gm. (approx.)	Isotopic ratio K^{39}/K^{41}	Per cent of K^{41}
1.	Rat	8	210	14.26	6.553
2.	Rat	4	220	14.20	6.579
3.	Rat	2	175	14.20	6.579
4	Rat	4	150	14.20	5.579
5.	Mouse	4	30	14.19	6.583

TABLE III: ANALYSIS OF POTASSIUM IN SKELETAL MUSCLE FROM TUMOR-BEARING RATS AND MICE

Sample no.	Species	No. of animals used	Average animal weight (without tumor) in gm. (approx.)	Average tumor weight in gm. (approx.)	Days after inoculation	Isotopic ratio K^{39}/K^{41}	Per cent of K^{41}
1.*	Rat	5	235	15.5	18	14.35	6.515
2.*	Rat	14	80	14.0	14	14.36	6.510
3.	Rat	2	150	30.0	16	14.34	6.519
4.*	Mouse	6	30	2.0	20	14.35	6.515

* Samples Nos. 1, 2, and 4 originated from the same animals as samples Nos. 1, 2, and 5 of Table I, respectively.

Table III; that of the resistant animals sample No. 3, Table II. The marked difference in the isotopic ratios indicates that the deviation was due to the development of the tumor, and not to the act of implantation.

The summary of results obtained with tumor tissue, and with muscle from normal and tumor-bearing animals, is presented in Fig. 1.

Discussion

The results, on the whole, indicate that potassium in tumor tissue and in muscle from tumor-bearing

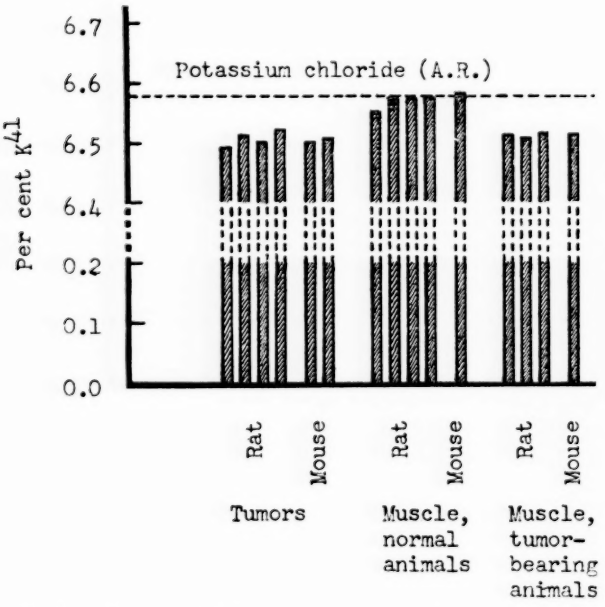

Fig. 1.—Content of K^{41} in potassium present in animal tumors (Jensen rat sarcoma, mouse sarcoma S 37) and in muscle from normal and tumor-bearing animals.

The height of each column indicates the percentage of K^{41} in potassium contained in one ash sample, while the dotted line indicates the percentage of K^{41} in mineral potassium. The columns are interrupted between 0.2 and 6.4 per cent K^{41}.

animals has a greater isotopic ratio, and consequently a smaller percentage of the heavy isotope, than mineral potassium and potassium in muscle and other tissues from normal animals. The difference is slight, but exceeds the experimental error of the mass spectrographic method.

For comparison it is interesting to note that a previous investigation of the radioactivity of potassium in tumor tissue (Jensen rat sarcoma) and in muscle from tumor-bearers failed to show any difference from normal (2). Since the radioactivity of potassium is due to the isotope K^{40}, the relative change to be expected should be only about half that which has been observed in respect to the isotope K^{41}. Deviations of this magnitude are, however, too small to be detected by the radioactive method of analysis used.

At present it is not possible to give a definite explanation of the isotopic shift herein described. However, the fact that a similar deviation was obtained with potassium in tumor tissue and in muscle from tumor-bearers appears to indicate a general and uniform change in the isotopic constitution of potassium in the tumor-bearing as compared with the normal animal. Corresponding deviations should therefore be expected in other solid tissues and especially in blood plasma. On the other hand, the difference in the isotopic ratios of tissue potassium and plasma potassium might under such conditions be similar in the normal and the tumor-bearing animal, and the same might apply to the mechanism responsible for this difference, as discussed in a previous paper (4). Whether, and to what extent, our supposition is correct, must remain a matter for further investigations.

Summary

The isotopic constitution of potassium in Jensen rat sarcoma and mouse sarcoma S 37 has been studied. Compared to mineral potassium as contained in ordinary potassium chloride (A.R.), a slight but definite increase of the isotopic ratio K^{39}/K^{41} was found, indicating a corresponding decrease in the percentage of the heavy isotope K^{41}. Since potassium in bone including marrow and blood plasma (from the rat) has shown similar deviations in the opposite direction, and as potassium in other normal tissues usually showed no deviation, it appears that the isotopic constitution of potassium in normal and tumor tissue is appreciably different. Comparison was also made of the potassium in muscle from normal and tumor-bearing rats and mice. In muscle from normal animals the isotopic ratio was generally the same as that of mineral potassium; but in tumor-bearing animals a deviation was observed similar to that found with tumor tissue.

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Xanthine Oxidase (Dehydrogenase) Activity in Livers of Mice of Cancer-Susceptible and Cancer-Resistant Strains*

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Introduction

There is a great variation in different species regarding susceptibility to both spontaneous carcinoma and tumors induced by numerous carcinogenic agents. Even within the same species, strains have been developed which differ significantly in intrinsic susceptibility and resistance to spontaneous tumors of several types (16, 17, 18). The difference in these strains is not limited to spontaneous mammary carcinoma, the most frequently occurring neoplasm in mice, but is observed also for tumors induced by methylcholanthrene (5). The JK strain, while not completely resistant, develops methylcholanthrene-induced tumors much more slowly than do mice of other inbred strains. Since the amount and potency of the injected carinogen were equal in all mice subjected to the treatment, the difference in rate of development of tumor represents different degrees of resistance (or susceptibility) to the same carcinogenic stimulus. It was thought that a systemic biochemical, physiological, or even morphological study and comparison of a highly resistant-to-cancer strain (JK) and a highly susceptible strain (C3H) might lead to the discovery of differences that could be correlated with differences in susceptibility. Since this study was limited to mice of the C3H and JK strains, the term "cancer-susceptible," as used in this paper, will have reference to (a) the very high incidence of mammary carcinoma observed in C3H mice at a relatively early age (8 to 10 months) as contrasted with the low incidence in mice of the JK strain, (b) the relatively short latent period for the development of methylcholanthrene-induced tumors as compared with the latent or induction period in mice of the JK strain. The term "cancer-resistant" will be applied to mice of the JK strain in reference to the low mammary carcinoma incidence and the relatively long induction period for methylcholanthrene-induced tumors.

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While the term "xanthine oxidase" is used throughout this paper, it is recognized that xanthine dehydrogenase would, perhaps, be a more accurate designation. However, the enzyme is more widely recognized and referred to as xanthine oxidase (1, 3, 8, 9, 10, 13). It is also used in reports that utilize anaerobic technic for measurement (12). As recently as 1938, it was suggested that the term Shardinger enzyme be dropped and the enzyme be called xanthine oxidase, even when its aldehyde activity is being discussed (3).

Many considerations led to the investigation of the possible relationship of xanthine oxidase activity to cancer susceptibility. It had been shown that growth of tumors of the mammary gland in mice could be inhibited by the administration of the proper dose of heptaldehyde (19, 20, 21). This has been confirmed by Boyland (4) who states that heptaldehyde, when given to mice, has some inhibitory action on the growth of both spontaneous carcinomas and grafted sarcomas. He also has been able to show that induced tumors, like grafted tumors, are less susceptible to the action of heptaldehyde than are spontaneous mammary carcinomas. In addition, he claims that a similar inhibitory effect on tumors is produced by dicarboxylic acids (particularly malonic acid) which are possible metabolic products of heptaldehyde. Mice of the cancer-resistant JK strain were also found to tolerate doses of salicylaldehyde that were very toxic in mice of the cancer-susceptible C3H strain (22). It was therefore desirable to investigate the mechanisms capable of acting on aldehydes after injection.

There was much evidence to indicate that xanthine oxidase (xanthine dehydrogenase), the Shardinger enzyme, liver dehydrogenase, and aldehydrase were identical and capable of oxidizing not only purines but also aldehydes (3, 7, 8). The controversy with regard to the identity of the xanthine oxidase, the Shardinger enzyme, milk dehydrogenase, and liver dehydrogenase was recently investigated (3) to settle this particular point. Most of the existing evidence in favor of identity was confirmed. Experiments purporting to demonstrate the existence of two separate enzymes could not be repeated or were shown either to have another explanation or to be insufficiently controlled (3). Since an adequate discussion of this has appeared recently (3), it will not be repeated here. However, it should be mentioned in this connection that in the preliminary and early stages of the experiments reported here, acetaldehyde, salicylaldehyde, and heptaldehyde were employed as substrates. The results with these aldehydes were similar and comparable to the later more quantitative results with xanthine as a substrate. According to Green (12), the oxidation reduction potentials of the hypoxanthine-xanthine systems and the xanthine-uric acid

systems controlled by the enzyme are the most negative observed in animal tissues. Thus it may be seen that xanthine oxidase may influence: (a) elimination of nucleoprotein constituents: (b) aldehyde tolerance: (c) other enzymes by virtue of the very negative oxidation reduction potential (11, 23). The relationship to the purine bases and nucleoproteins was in itself sufficient to stimulate investigation of the enzyme.

Xanthine oxidase has been found in greatest abundance in liver and milk. Both sources have been used for extraction of enzyme (1, 9, 13, 14, 25). It is also abundant in lactating mammary glands of mice (Figge and Strong, unpublished). One of the most unusual facts regarding this enzyme is that high concentrations of either substrate or end product inhibits the enzyme. Dixon and Thurlow (10) at first reported that xanthine oxidase was not inhibited by cyanide. However, in 1936, Dixon and Keilin (8) discovered several remarkable features regarding inhibitions of xanthine oxidase. The enzyme was irreversibly inactivated by cyanide in the absence of purines, but not in their presence. The end product, uric acid, also protects the enzyme from cyanide. Hypoxanthine protects the enzyme only in the presence of a hydrogen acceptor. Peroxide destroys the enzyme. Catalase protects it during aerobic oxidations (8).

It was necessary to take many of these facts into account in order to determine the enzyme activity in mouse livers. At first, an attempt was made to extract the enzyme according to the methods of Harrison (14) and Wieland and Frage (25). This was abandoned because such extraction procedures, even when standardized, did not seem reliable quantitatively. Next an attempt was made to determine the enzyme by grinding liver in sand and adding water or buffer. This whole liver brei was tested for enzyme activity. Some qualitative results were obtained in this way, but the inhibitory effects of naturally occurring substrate and end products gave rise to values that were variable. Since all the known substrates and end products that might act as inhibitors consist of relatively small molecules, this difficulty was solved by simply dialyzing them out of the liver preparation. The results obtained by this method were uniform and reproducible. Striking differences were observed in the xanthine oxidase activity of livers from the two strains of mice.

Experimental Methods and Materials

The mice were from Strong's inbred C3H and JK strains (16, 18). The ages of all mice were known and a wide variation in ages (25 to 575 days) was included in the analysis. Mice were also chosen from both sexes. All mice had been maintained on Nurishmix diet. Including preliminary experiments, 70 mouse livers were analyzed. Quantitative data were obtained for 43 normal mice.

Each mouse was weighed, then killed by a blow and immediately decapitated. The liver was quickly removed, rinsed, dried with filter paper, and weighed. The entire liver (gall bladder removed) was placed in a glass mortar. Washed and ignited sea sand for grinding was also weighed. The amount of sand added was arbitrarily one-half the weight of the liver. The liver and the sand were then ground for 5 minutes. During the grinding, 3 volumes (based on total weight of liver) of distilled water were added. Five drops of toluene were also added at this time in order to protect the xanthine oxidase enzyme from deterioration. The sand-liver-water mixture was then poured into a dialysis bag (cellophane Visking) and three more volumes of water were added in 3 portions to rinse the liver and sand into the dialysis bag. The ground liver was then dialyzed for 3 to 4 hours in running tap water in a vessel so arranged that the movement of water kept the bag and its contents in continual motion. After this, the bag was placed in a pint glass jar containing distilled water and 5 drops of toluene. It was allowed to dialyze for 12 hours in a refrigerator.

The contents of the cellophane bag were poured into a weighed centrifuge tube. The bag was rinsed 3 times with distilled water and the water content of the preparation was adjusted by weighing so that the total weight of water added was 10 times the weight of the liver. Each cc. of such an extract thus represented 100 mgm. of liver. After centrifugation the supernatant fluid was analyzed for enzyme activity. In the preliminary experiments, it was ascertained that the sand did not absorb appreciable amounts of enzyme, and that the precipitated liver material was not more active than the supernatant fluid. With few exceptions, each mouse liver was tested for enzyme activity separately.

The Thunberg technic of methylene blue reduction in an oxygen-free medium for estimating enzyme activity was essentially the same as that used by Wieland (24) and Green and Dixon (13). With proper precautions this method gave results that agreed closely with manometric methods (7). Four tubes were used for each determination. The substances that were introduced into the Thunberg tube are indicated as follows:

Substances added:	Tube number			
To tube	1	2	3	4
Methylene blue N/1000	1 cc.	1 cc.	1 cc.	1 cc.
Phosphate buffer M/15 pH 7.6	2.6 cc.	2.6 cc.	2.6 cc.	1.6 cc.
Liver preparation	1 cc.	1 cc.	1 cc.	2 cc.
To stopper				
Xanthine 1/200 M	....	0.2 cc.	0.4 cc.	0.4 cc.
Distilled water	0.4 cc.	0.2 cc.	....	....

It should be noted that the substrate was placed in the stopper and not mixed until after evacuation and establishment of temperature equilibrium. No substrate was added to tube 1. This was done to make certain that the dialysis had completely removed all natural substrate. The concentrations of substrate

and enzyme were varied in tubes 2, 3, and 4. After 20 determinations it was apparent that tubes 2, 3, and 4 always gave the same values for enzyme activity, thus indicating optimal conditions for the reduction of methylene blue by xanthine oxidase in the presence of xanthine. When this was ascertained only tube 4 was used for a determination.

To obtain quantitative results with this technic, it was necessary to remove completely all oxygen. This was done by evacuating the air with a water pump until the solution began to boil. The tube was then filled with prepurified nitrogen.[1] This procedure was repeated 5 times, and after the final evacuation enough nitrogen was admitted to bring the pressure to 64 cm. mercury. It was found that if the tubes were left highly evacuated there was a greater tendency to draw oxygen into the tube even though the stoppers had been well greased.

After evacuation, the tubes were placed in a constant temperature oven with a glass front. At least one-half hour was allowed for the tubes to reach temperature equilibrium (37° C.). A control tube of water with a thermometer bulb in the water was used to make certain that the proper temperature was established before the contents of the tubes and stoppers were mixed. The tubes were held in a device that permitted the tilting and mixing of the contents of all tubes and stoppers simultaneously. This device could also be rotated without opening the glass door of the oven, so that as many as 12 tubes could be observed with minimum temperature variation.

The units of enzyme activity per gram of liver (wet weight) were calculated according to the method of Wieland (24). Arbitrarily, a unit of enzyme activity reduced 1 cc. of 0.001 N methylene blue in 5 minutes at pH 8 and at a temperature of 37° C. Accordingly, the number of units of enzyme activity for any given preparation was calculated as follows $\frac{5}{\textit{decolorization time (for 200 mgm.)}}$. This gave the number of enzyme activity units per 2 cc. of enzyme preparation. Since this represented only 200 mgm. of liver, the number of activity units per gm. was calculated by multiplying this number by 5.

Results

Table I shows the decolorization times for the liver preparations of the individual animals together with other pertinent data. The animals are arranged in Table I according to strain, sex, and age. An inspection of this table shows that there was a marked difference in the decolorization time in the livers of mice of the two strains (JK and C3H). The average time required for 2 cc. of the JK liver preparation to reduce the methylene blue was 16 minutes, while 2 cc. of the C3H liver preparation required 33 minutes, or over twice as long to bring about the same result. The control tubes containing liver preparation, buffer, and methylene blue, but no xanthine, did not decolorize the methylene blue in less than 5 or 6 hours.

Table I: Xanthine Oxidase Activity of Livers of Cancer-Susceptible (C3H) Strain and Cancer-Resistant (JK) Strain

Sex	Weight, grams	Age, days	Decolorization time, min.	Enzyme activity units per gm. liver
		C3H		
F	8.1	30	42	0.60
F	8.1	30	42	0.60
F	11.6	36	25.5	0.98
F	16.9	59	25.5	0.98
F	17.0	59	36	0.70
F	20.8	185	36	0.70
F	25.7	185	34	0.74
F	23.6	215	47	0.53
F	23.4	275	67	0.37
M	5.0	18	33	0.75
M	5.0	18	33	0.75
M	13.0	36	25	1.00
M	25.0	360	42	0.60
M	22.5	367	26	0.96
M	25.0	370	29	0.86
M	22.4	377	23	1.09
M	23.5	377	29	0.86
M	23.6	377	29	0.86
M	24.4	377	29	0.86
M	24.0	405	29	1.04
M	25.4	405	20	1.25
			Av. 33	Av. 0.81
		JK		
F	10.0	47	13	1.92
F	12.8	66	23	1.09
F	13.0	66	25	1.00
F	15.5	105	25	1.00
F	23.1	196	9	2.78
F	20.4	417	20	1.25
F	20.7	505	14	1.79
F	18.2	545	19	1.32
M	8.0	25	17	1.47
M	8.7	25	17	1.47
M	8.6	25	17	1.47
M	8.5	25	17	1.47
M	21.0	138	14	1.79
M	20.7	138	16	1.56
M	24.4	195	12	2.09
M	19.6	205	16	1.56
M	19.5	230	16	1.56
M	20.4	230	16	1.56
M	20.5	230	16	1.56
M	20.5	322	14	1.79
M	21.7	570	9	2.78
M	18.5	575	14	1.79
			Av. 16	Av. 1.64

[1] From the Air Reduction Sales Co.

This reduction over a long period in the absence of substrate is probably caused by bacteria (6) rather than xanthine oxidase activity.

Double and triple determinations on many of the preparations gave decolorization time values that agreed within 2 minutes, so that very little of the observed variation can be due to the method employed. Using 2 cc. of liver preparation the maximum decolorization time for the C3H series was 67 minutes and the minimum was 20 minutes. The maximum for the JK series was 25 minutes and the minimum decolorization time was 8 minutes. It should be noted, however, that only two of the C3H liver preparations decolorized the methylene blue in less than 25 minutes (23 and 20 minutes). With the exception of these two cases there is no overlap in the data on decolorization time for liver preparations of mice of the two strains.

No significant difference was noted with regard to sex in the JK series. The average decolorization time for the C3H females was 39 minutes, while the average for the males was only 29 minutes. A sex difference in this strain is thus indicated, but more determinations are necessary to establish this point.

The enzyme activity per gm. of mouse liver was calculated according to the method of Wieland (24). These are listed in Table I and depicted graphically in Fig. 1. The average number of xanthine oxidase activity units for the JK (cancer-resistant mice) is 1.64 per gm. of liver. The average number of activity units for the C3H (cancer-susceptible mice) is about one-half this or 0.81.

The livers of C3H mice are generally heavier than livers of the JK strain, but even if the xanthine oxidase activity is calculated on the basis of total liver weight, the JK strain of resistant mice have a greater number of enzyme activity units. The same relationship holds if this is calculated and stated in terms of xanthine oxidase activity units per 20 gm. of body weight of mouse.

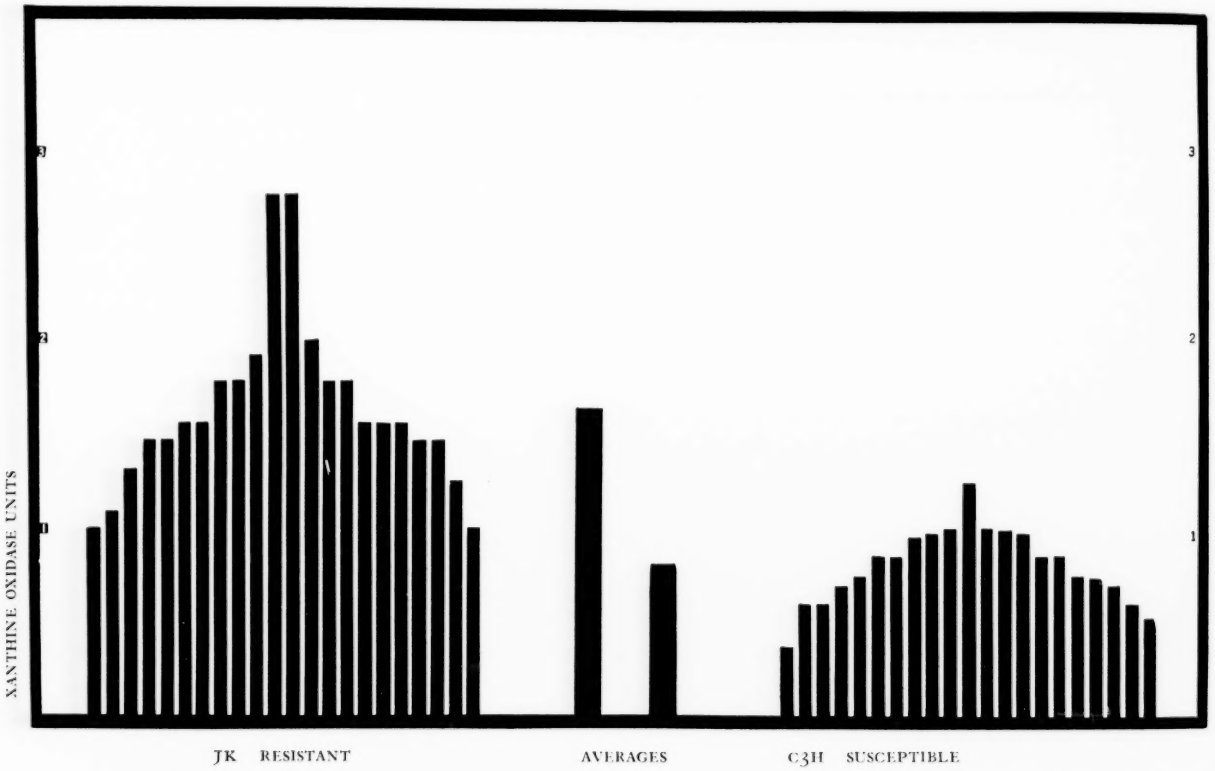

FIG. 1.—The narrow vertical bars represent xanthine oxidase activity units in individual mouse livers of the JK cancer-resistant strain of mice and in C3H cancer-susceptible strain of mice. They are arranged to show the variation. The wide bars represent averages.

DISCUSSION

The difference in the decolorization time (rate of reduction of methylene blue) indicates a difference in xanthine oxidase (dehydrogenase) activity in the livers of mice of the two strains used in this investigation. However, it may be possible to explain the observed difference in decolorization time in other ways. The dialyzed preparations used in these experiments probably contained several other liver enzymes. Throughout this paper, the observed differences in decolorization time have been interpreted and referred to as

differences in xanthine oxidase activity (not xanthine oxidase concentration). Whether this difference in xanthine oxidase activity is due to different concentrations of xanthine oxidase, other enzymes, or substances is a problem for further research.

Extreme variability in rate of reduction of methylene blue was observed in the undialyzed preparations of liver. On the other hand, the dialyzed liver preparations gave uniform and reproducible results. It was apparent that the rate, in undialyzed preparations, was merely a function of the concentration of substrate and end products. Low concentration of substrate gave rise to low enzyme activity, while high concentrations of either substrate or end products completely inhibited the reduction of methylene blue. The numerous experiments involving reduction of dyes or oxygen consumption in undialyzed whole liver preparations would, therefore, seem to lack significance.

The relationship of this observed difference in xanthine oxidase activity to cancer susceptibility is as yet somewhat obscure. The present observation on greater xanthine oxidase activity would indicate that the JK mouse (cancer-resistant) has a more efficient mechanism for destroying some of the breakdown products of nucleoproteins than the C3H mouse (cancer-susceptible) has. If some of the purine bases which act as substrates for xanthine oxidase are also used in the synthesis of nucleoproteins, then the JK mouse also has a more efficient mechanism for destroying the precursors of the nucleoproteins. Since xanthine oxidase also oxidizes aldehydes, the observed difference in activities in the two strains of mice falls in line with the studies on salicylaldehyde toxicity tolerance (22).

Xanthine oxidase from milk has been shown to be an alloxazine proteid or flavin dinucleotide, somewhat similar to "yellow enzyme" (1). It is of interest in this connection that riboflavin plus casein inhibited butter yellow cancer in rats (15). Some attempts have been made to influence cancer by means of liver feeding (2). Liver diets contain not only xanthine oxidase itself, but also considerable amounts of riboflavin and perhaps other substances that may be necessary for the synthesis of the enzyme.

The variability observed in both strains is approximately what one would expect in any biological experiment. In regard to this variation it should be remembered that while a marked difference with regard to mammary carcinoma susceptibility exists between the two strains, there is, however, some variation in the animals within either strain. This variation is not limited to susceptibility to methylcholanthrene-induced tumors but holds also for spontaneous tumors of the mammary gland. The variability in xanthine oxidase activity of the liver thus corresponds roughly to the variability in susceptibility or resistance to cancer.

The criticism that enzymes cannot be quantitatively extracted cannot be applied to the method chosen for this work. No attempt was made to extract the enzyme. The substrate and end products were merely dialyzed out and the optimum concentration of xanthine added to permit the reaction to take place. Since the decolorization of methylene blue did not occur when xanthine was not added, it is justifiable to refer to the activity which occurs after the addition of xanthine as xanthine oxidase activity.

Stripped of all interpretation and speculation, the fact remains that when mouse livers of the C3H and JK strains were treated in precisely the same way, the JK liver preparations in the presence of added xanthine reduced methylene blue twice as fast as C3H liver preparations.

Summary

1. A method for determining the xanthine activity of liver was devised. The essential and new feature of this method was the removal of naturally occurring substrates and end products by dialysis.

2. Xanthine oxidase activity was determined quantitatively in livers of 21 C3H cancer-susceptible and 22 JK cancer-resistant mice.

3. The C3H livers averaged 0.81 xanthine oxidase activity units per gm. of liver. The JK livers averaged 1.64 xanthine oxidase activity units per gm. of liver. These values correspond to a methylene blue reduction time of 33 minutes for C3H and 16 minutes for JK livers.

4. A discussion of a possible correlation between xanthine oxidase activity and cancer susceptibility is included.

5. The present investigation thus advances the previous work on (a) tolerance to salicylaldehyde as being a measure of cancer susceptibility and (b) heptylaldehyde as an inhibitory or stimulating influence on cancer of the mammary gland in mice depending upon the concentration of the material administered.

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Transplantation of Leukemia Arising in Hybrid Mice*

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It has been reported by Schweitzer and Furth (5) that leukemic cells arising in hybrid mice obtained by crossing mice of a low leukemia stock (Rf) and a high leukemia stock (Ak) could be transplanted into almost 100 per cent of mice of the high leukemia parent stock and F1 hybrids, and into a smaller per cent of other hybrid mice, but into none of the low leukemia parent stock. As far as transplantation was concerned, the leukemic cells of the hybrids behaved in a manner similar to leukemic cells arising in the high leukemia stock (5). Leukemic cells arising in hybrid mice treated with carcinogens did not behave in the above manner (2). Only 1 of 6 transmissible leukemias induced in hybrids resembled those arising spontaneously. Two could be grafted on both parent stocks (high leukemia and low leukemia) and 2 could be grafted on hybrids but on neither parental stock. The authors (Furth and Barnes) attempted to explain this difference by assuming that the cells of the leukemias induced by carcinogens are mutants.

Since the above findings (5) on transplantation of spontaneous leukemia arising in hybrids are at variance with the results obtained in transplantation of carcinoma of the mammary gland and normal splenic tissue (1, 7),[1] experiments similar to those of Schweitzer and Furth were carried out, using the high leukemia inbred F strain[2] (3) and the low leukemia CBA strain[3] (6) as the parent stocks. The purpose of the investigation was to determine whether leukemic cells of hybrids (F1, F2, and both backcross generations) between other high and low leukemia strains (F and CBA) are transplantable in a manner similar to that observed by Schweitzer and Furth for the Ak and Rf stocks, or whether the behavior of the leukemic cells might with our material be similar to that shown by the cells of mouse mammary cancer and normal splenic tissue.

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[1] Tissue from F1 hybrids can be grafted into F1 hybrid mice but into neither parent stock.

[2] The incidence of spontaneous leukemia in the F strain is 55 per cent.

[3] The incidence of spontaneous leukemia in the CBA strain is less than 1 per cent.

Materials, Methods, and Results

Pure strain F mice were crossed with CBA mice to obtain F1 hybrids. These were mated with each other to get F2 hybrids and were also backcrossed to the CBA and to the F strains. Cases of spontaneous leukemia have appeared in hybrids of all types. Leukemic cells were inoculated into pure strain F mice and CBA mice. In 4 cases leukemic cells of F1 hybrids were inoculated into F1 hybrid mice as well as into the pure parent stocks. Transplantation was carried out by the intraperitoneal inoculation of a suspension of leukemic cells (70,000,000 cells inoculated, cells taken from spleen in every case). Mice were not considered negative for leukemia until from 59 to 180 days following inoculation had elapsed. The results are summarized in Table I. The data presented demonstrate that the leukemias arising in F1 hybrids, F2 hybrids, and backcrosses to the CBA strain could not be transplanted into either parent strain; the leukemic cells of F1 hybrids were successfully transplanted into F1 hybrid mice, and in 2 cases out of 4 (1 questionable) mice of a leukemic strain were susceptible to leukemic cells arising in backcrosses to this strain.

In every instance in this laboratory successful transplantation was accomplished when the leukemic cells from a spontaneous case in a mouse of pure strain were inoculated into normal mice of the same strain (3, 4)—12 cases in the F strain, 4 cases in the CBA strain. Even in the first transfer generation leukemia usually appeared within 6 weeks after inoculation. Approximately 5,000 mice have been inoculated with leukemic cells in succeeding transfer generations with uniformly positive results; following repeated transplantation the latent period preceding the appearance of leukemia was decreased. One hundred per cent of F1 hybrid mice proved to be susceptible to the cells of a pure strain leukemia; approximately half of F2 hybrids were susceptible.

Conclusions

Leukemic cells arising in hybrids between the high leukemia F strain and low leukemia CBA strain were transplantable in a manner similar to that found for mammary cancer and normal splenic tissue of hybrid mice; that is, the leukemic cells of F1 hybrids grew

TABLE I: SUMMARY OF RESULTS FOLLOWING INOCULATION INTO F MICE, CBA MICE, AND F1 HYBRIDS OF LEUKEMIC CELLS ARISING IN VARIOUS TYPES OF HYBRIDS

Spontaneous leukemia in	Transplanted into F strain mice *	Transplanted into CBA mice †	Transplanted into F1 hybrids	Days before mice considered negative
F1 hybrid F♀ × CBA♂	2 mice–negative	2 mice–negative	1 mouse–positive	106
F1 hybrid F♂ × CBA♀	2 mice–negative	2 mice–negative	1 mouse–positive	106
F1 hybrid F♀ × CBA♂	2 mice–negative	2 mice–negative	1 mouse–positive	106
F1 hybrid ‡ F♀ × CBA♂	2 mice–negative	2 mice–negative	1 mouse–positive	106
F1 hybrid F♂ × CBA♀	3 mice–negative	3 mice–negative	.....	59
Totals	11 mice–0 positive	11 mice–0 positive	4 mice–4 positive	
Backcross F♂ × (F♀ × CBA♂)♀	4 mice–positive	4 mice–negative	.....	100
Backcross F♀ × (F♀ × CBA♂)♀	2 mice–1 positive? 1 negative	.....	.....	88
Backcross F♂ × (F♀ × CBA♂)♀	3 mice–negative	.....	.....	75
Backcross F♂ × (F♀ × CBA♂)♀	3 mice–negative	.....	.....	165
Totals	12 mice–5 positive 7 negative	4 mice–0 positive		
Backcross CBA♂ × (CBA♂ × F♀)♀	4 mice–negative	4 mice–negative	.....	180
Backcross CBA♀ × (CBA♀ × F♂)♂	3 mice-negative	3 mice-negative	.....	71
Backcross CBA♀ × (CBA♂ × F♀)♂	3 mice-negative	.....	.....	75
Totals	10 mice–0 positive	7 mice–0 positive		
F2 hybrid (F♀ × CBA♂) §	4 mice–negative	4 mice–negative	.....	162
F2 hybrid (F♀ × CBA♂) §	3 mice-negative	3 mice-negative	.....	91
F2 hybrid (F♀ × CBA♂) §	3 mice-negative	3 mice-negative	.....	165
Totals	10 mice–0 positive	10 mice–0 positive		

* Susceptible to spontaneous leukemia.
† Resistant to spontaneous leukemia.
‡ Myelogenous leukemia. Others were lymphatic leukemia.
§ Both male and female parents were F1 hybrids obtained by crossing an F♀ with a CBA♂.

in neither parent stock but in all F1 hybrids. Leukemic cells of F2 hybrids grew in neither parent stock; leukemic cells from a small per cent of backcross mice grew in the parent stock to which the F1 hybrid parent had been backcrossed.

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The Effect of Testosterone Propionate on Mammary Tumors in Mice of the C3H Strain

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A relationship between estrogenic activity and the production of tumors of the mammary gland has been clearly demonstrated in various strains of mice. In view of the inhibitory action of testosterone on estrus, lactation, and the vaginal response to estrone in mice (4, 5), the possibility of affecting spontaneous tumor formation by inhibition of estrogenic activity seemed worthy of further investigation. In 1936, Lacassagne (2) reported that injections of testosterone had not altered the incidence of spontaneous mammary cancer in females of a high cancer strain, but the amount of hormone used was small.

This report presents the end results of long-continued administration of testosterone propionate on the development of spontaneous mammary carcinoma in female mice of a high cancer strain (a) when injections were begun by the 5th week of life, and (b) when injections were given to older females with a history of normal breeding. It also includes observations on the failure of testosterone to affect the growth rate of mammary carcinoma in mice when administered after the tumor is of palpable size.

The experiments were started in the spring of 1938 and extended over 2 years. They were well under way when the report of Nathanson and Andervont (3) was published, but since our experiments differed in some respects from theirs, it was decided to continue them.

Nathanson and Andervont (3) gave testosterone for a period of 4 months to C3H female mice, 4½ months of age or younger, which had previously borne one litter. Thirty per cent developed mammary carcinoma within 4 months after injections of the hormone were started, and died before the 11th month of age, with only a single tumor. Ten were still alive and without tumors at 16 months. The fate of the surviving mice has not been reported. All of the controls had developed at least one, and many of them multiple, tumors by the 11th month of life. The authors believe that the administration of testosterone prevented the development of tumors, "provided there were none present when the treatment became effective." They assume that those tumors which did develop were microscopic in size when treatment was started.

Materials and Methods

In the experiments reported here, female mice of the C3H strain [1] were likewise used. In the first experiment, 14 females received subcutaneous injections of testosterone [2] over a period of 45 weeks. Ages at the first injection ranged from less than one week to 5 weeks. The testosterone was diluted with olive oil so that each 0.05 cc. contained 0.5 mgm. of the hormone. For the first 4 weeks, 11 of the mice received 3 injections weekly, each containing 0.5 mgm. of the hormone. For the following 41 weeks, they received one injection containing 1.0 mgm. weekly. These mice were from 18 to 34 days of age at their first injection. The remaining 3 mice were given their first injection containing 0.1 mgm. of hormone at 2 days of age. After the first week, the amount was increased to 0.5 mgm. three times weekly for 3 weeks, and then reduced to 1.0 mgm. once a week for the remaining 41 weeks. These mice exhibited slight modifications of the external genitalia. None of the treated females became pregnant although a male of proven fertility was kept with them over long periods of time.

[1] The author is indebted to Dr. H. B. Andervont for the original C3H mice from which our strain has been developed. These consisted of two litters with 3 females and a sib male in each. The stock has been continued by brother-to-sister mating. Among the descendents of the two litters, 91 per cent of 300 bred females have developed mammary gland cancer, which is the same (91.37 per cent) as Andervont's (personal communication) for bred females of this strain in his laboratory. Virgin females, however, have not shown the expected high incidence of such tumors. In Andervont's stock (1), the cancer incidence is even higher (97.43 per cent) in virgin females. In our substrain, however, wide variation in tumor incidence in virgin females has been encountered among the offspring of the 6 original females. Unfortunately, the numbers are small, and as our present colony is composed of descendants from only one of the original females, we are unable to investigate the matter further.

[2] The crystalline propionic ester of testosterone in oil, Oreton, prepared by the Schering Corporation. We are indebted to Dr. Erwin Schwenk for the supply used in these experiments.

Injections were discontinued when the mice were 45 to 50 weeks old, the average age at which virgin females develop mammary tumors in this strain. They had thus received testosterone injections throughout the period during which tumors arise in the majority of such females.

Controls.—Thirty-eight virgin females from the same sublines as the treated females have been used as controls on which to base expectancy of tumor incidence, age at appearance of tumors, and age at death of noncancerous individuals. Of these, 18, or 47 per cent, developed tumors of the mammary gland at an average age of 12.0 months. The average age at death of noncancerous mice was 19.7 months.

Results

Two treated mice died before reaching tumor age. Of the remaining 12, 3 developed adenocarcinoma of the mammary gland at 15, 22, and 24 months, 3, 10, and 12 months, respectively, after cessation of treatment. Injections of testosterone had been started in one of these at 18 days of age and in the other 2 at 34 days. In 2 of them, both ovaries were almost completely replaced by large cysts filled with clear fluid. Two mice developed tumors at other sites, one a sarcoma adjacent to an old oil abscess, at 27 months, and the other a primary liver cell tumor, discovered at autopsy at 27 months. Such tumors are known to occur in mice of this strain and have no bearing on the present problem. The remaining 7 mice died or were killed for autopsy at an average age of 21.7 months. Four females, in addition to the 2 with mammary tumors mentioned above, had cysts almost replacing one or both ovaries, leaving only a thin rim of ovarian tissue. Similar cysts were found in 3 of the control virgins, and have been found occasionally in bred females of this strain. In one treated mouse, a large cyst was found in the left adrenal gland.

Whole mounts of mammary glands were made from 8 treated females and from 10 control virgins of comparable age. The control glands are characteristically composed of extremely wide, cystic-appearing ducts, with no hyperplasia of the lining epithelium. Many of the ducts end in club-shaped or bulbous enlargements. In some glands, there are also a few narrow, branching ducts with grapelike clusters of alveoli and occasional isolated and localized areas of alveolar proliferation. Cellular infiltration is common around the alveoli, and rarely ducts and alveoli are surrounded by a cloud of lymphocytes.

Glands from testosterone-treated females are variable in appearance. Moderately wide, cystic ducts are present in all cases, but in none is the widening as exaggerated or extreme as in the controls. In almost all cases, in addition to the widened ducts, there are also narrow, branching ducts with small clusters of alveoli. In 2 mice, the glands are predominately of the narrow, branching type. Small, localized areas of alveolar proliferation are present in several cases, and periductal infiltration is common although variable in amount.

In view of Nathanson and Andervont's report (3) that bred females (having had one litter which was removed at birth) injected with testosterone over a period of 4 months had a lower tumor incidence than control females, a study was undertaken of the effect of testosterone on a group of bred females which had suckled their young. Thirty-two females which had borne and nursed successfully 1 to 3 litters were divided into two groups with sister litter mates of similar breeding history in each group. Ages ranged from 4 to 8 months, with an average age of 5½ months at beginning of treatment. Eight females in each group had borne 1 litter, 7 had borne 2, and one had borne 3. Injections of 1.0 mgm. of testosterone were given once a week over a period of 4 months, or until the development of tumors. All females, both control and experimental, developed tumors, at an average age of 9 months for the controls and 9.6 months for the treated. Thus, in older females of normal breeding history, injections of this amount of testosterone (which was smaller by one-third than the amount given by Nathanson and Andervont) were ineffective in lowering tumor incidence and in delaying the appearance of tumors to any appreciable extent.

An attempt to influence the growth rate of tumors of palpable size was without effect, as previously reported by Nathanson and Andervont (3). Ten females were used in this study. Biopsy was done on each tumor and treatment started the second day following operation. Injections of 1.0 mgm. of testosterone were made subcutaneously on the side opposite the tumor, and were given three times weekly, or until the appearance of a second tumor. All tumors grew progressively and 3 of the mice developed a second tumor during the period of treatment.

Discussion

Testosterone was administered to C3H female mice from the 2nd to the 12th month of life, the period during which a majority of untreated females of this strain, virgin as well as bred, usually develop tumors. None developed tumors during the period of treatment. Three (25 per cent) of those living to cancer age developed tumors of the mammary gland at an average age of 20 months. Eighteen controls (47 per cent) developed tumors at an average age of 12.0 months. Thus, tumor incidence has been reduced by testosterone.

Because of the small number of mice involved, it is difficult to judge whether or not the age at macroscopic appearance of tumors has actually been increased. The 3 tumors among testosterone-treated individuals developed at 15, 22, and 24 months. Among the 18 control mice with tumors, 6 were seen for the first time after 12 months of age, at 13, 14 (2 individuals), 15, 16, and 24 months. Thus, the late age at which tumors developed in the treated mice may be a chance occurrence. The results, however, are interesting in view of the suggestion of Nathanson and Andervont that administration of testosterone propionate will prevent the development of tumors provided there was none present when treatment became effective. If this is the case, then tumors were in existence in the 3 individuals of the present experiment before 5 weeks of age. Since treatment with testosterone apparently does not influence the growth rate of tumors already established, the advanced age at macroscopic appearance of tumors can hardly be attributed to a retarding effect of the hormone. It seems reasonable to believe, therefore, that these tumors arose after cessation of treatment, when there may possibly have been renewed estrogenic activity stimulated by a pituitary reaction after the withdrawal of the male hormone.

Summary and Conclusions

1. Injections of testosterone propionate to virgin female mice of the C3H strain from the 2nd to the 12th month of life lowers the incidence of spontaneous mammary gland tumors, and possibly increases the age at macroscopic appearance of tumors.

2. The incidence and age at appearance of tumors in females of normal breeding history are not influenced by testosterone in the amounts used in these experiments.

3. Injections of testosterone do not inhibit growth of mammary gland tumors once they have reached macroscopic size.

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Studies on the Effect of Foster Nursing and its Relation to the Development of Mammary Carcinoma in the Mouse *

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The discovery of an extrachromosomal effect, which is approximately 10 times more powerful than any possible chromosomal influence in determining the incidence of mammary cancer in mice (5, 6, 8), has stimulated attempts to separate and evaluate the parts which these two influences play in the development of this type of tumor.

The general agreement among investigators as to the presence of the extrachromosomal stimulus leaves little doubt that this is a prime influence in determining whether or not mammary carcinoma will appear. There is less unanimity of opinion, however, as to whether or not the action of particulate cancer genes [1] is necessary to supplement and activate the extrachromosomal influence.

One of the reasons for this difference of opinion is the finding of a "residual amount" of mammary cancer in the most resistant strains of mice. None of the inbred stocks thus far developed has been proved to be free from this type of cancer. Because of this residual amount of tumor which appears in experiments designed to test the presence of particulate cancer genes in the chromatin, the presence of such cancer genes has never been satisfactorily proved or disproved.

It is the purpose of this paper, by reviewing some of the results obtained by us (Table I), to demonstrate that the ratios in which mammary tumors occur, in the several inbred strains and experimental crosses, may be reasonably explained upon the basis of the strength of the extrachromosomal stimulus which the mice receive through the mothers' or foster mothers' milk and the resistance of the various physiological systems [2] upon which the extrachromosomal stimulus reacts.

TABLE I: EFFECT OF EXTRACHROMOSOMAL STIMULUS UPON RATIO OF CANCER RATES IN MICE

Stock (Virgin females)	No. of animals	No. having mammary cancer	Percentage of cancer	Cancer rate per 10,000 population	Ratio of cancer rates
Dilute brown (dbr)	445	274	61.6	575	100
dbr—fostered to black females	108	25	23.1	132	23
dBF1 (dbr females × black male)	113	45	39.8	300	52
dBF2 (dBF1 female × dBF1 male)	664	236	35.5	256	44
C57—fostered to dbr females	98	9	9.2	60	10
BdF1 (black female × dbr male)	379	8	2.2	11.7	2
BdF1—fostered to dbr females	93	60	64.5	339	59
dBF1—backcrossed to black, 8 gen	180	0	0.0	0	0
BdF1—backcrossed to dbr, 8 gen	150	3	2.0	11	2

EFFECT OF THE EXTRACHROMOSOMAL INFLUENCE ON RECEPTIVE AND RESISTANT ANIMALS

It is a well-established fact that the milk stimulus which is transmitted by the females of a number of inbred strains (1, 2, 3, 4) stimulates their young to have tumors in proportions strikingly similar to those which appear in the parent generations. It is equally true that if the very resistant strains transmit a milk stimulus, they do so in concentrations which are not strong enough to produce tumors in their young in appreciable amounts.

That this milk stimulus is present in the dilute brown strain is hardly to be questioned. That it is present in the C57 black stock is indicated by the

* Read at the 34th Annual Meeting, American Association for Cancer Research, Inc., Chicago, Illinois, April 15, 1941.

[1] The expression "particulate genes" is employed in the sense used by Mendel, Morgan, and others.

[2] If the genetic theory is accepted, each individual is a physiological system the make-up of which is primarily determined by its genetic constitution. "Genetic constitution" is here interpreted as being the interaction of the total number of genes which the individual inherits.

behavior of animals of this strain in having an occasional tumor, and by the finding that graded amounts of tumor found in other stocks have a high correlation with the concentration of the milk stimulus which they transmit (7).

When the dilute brown and C57 strains are crossed reciprocally, the first generation hybrids develop tumors in degrees which are significantly different (6, 8). This difference may be explained upon the basis of the reaction of different concentrations of stimulus working upon the same genetic constitution just as readily and as reasonably as it may be explained by reasoning that the small amount of tumor arising in the BdF1 cross is that fraction of the total amount of tumor which is directly attributable to the presence of particulate cancer genes.

The reaction of the dilute brown physiology in the absence or dilution of the normal stimulus received by this stock and the reaction of the black physiology, in the presence of the concentrated dilute brown stimulus indicates:

1. The stimulus is either markedly less in the black strain than in the dilute brown, or

2. The resistance of the black physiology to the dilute brown stimulus is greater than that of the dilute browns to the same stimulus.

The ratios of tumors in these stocks are shown in Table II.

Table II: Ratios of Tumors in Dilute Brown and C57 Black Strains

Stock	No. of mice	Stimulus	Ratio of cancer
Dilute brown	445	Dilute brown	100
Dilute brown	108	Black	23 *
Black	200	Black	1 plus
Black	98	Dilute brown	10

* The figure 23 for the dilute brown fostered to black is probably high, because of the possibility that these animals received some stimulus from their mothers before being fostered. van Gulik and Korteweg (9) found less than half as many tumors in a duplication of this experiment.

The dilute brown physiological system when subjected to the dilute brown stimulus, develops 5 to 10 times as much tumor as it does when subjected to the milk stimulus transmitted by the black females. Two physiologies of markedly different resistance react in significantly different manner to the same concentration of a stimulus.

These data are construed as indicating that:

1. The dilute brown stimulus is 5 to 10 times more active than that transmitted by the black females.

2. The blacks are 5 to 10 times more resistant to the extrachromosomal stimulus than are the dilute browns.

Effects Observed in Hybrids Between Resistant and Receptive Strains

The effect of foster nursing upon the ratio of cancer in certain hybrids of the dilute brown and C57 strains is shown in Table III.

In reciprocal hybrids between the C57 black and dilute brown strains, we find that the first generation produced by mating dilute brown females to black males, which nursed their own mothers, has a cancer ratio of 52. The hybrids produced by mating black females to dilute brown males have a cancer ratio of 2. These latter animals, when foster nursed to dilute brown females, give a ratio of 59. These data are offered as further evidence that the ratio of cancer observed in the parent stocks is directly dependent upon the concentration of the stimulus received. These hybrid physiologies, regardless of the direction in which the cross is made, give comparable amounts of tumor when subjected to the same strength of stimulus.

Table III: Effect of Foster Nursing Stimulus upon the Ratio of Cancer in Hybrids

Cross	No. of mice	Foster nursing stimulus	Ratio of cancer
Dilute brown female × black male	113	dbr	52
Black female × dilute brown male	379	black	2
Black female × dilute brown male	93	dbr	59

These findings indicate that the amount of mammary carcinoma occurring in a stock or cross, may be explained on the basis of:

1. The concentration of the extrachromosomal stimulus received.

2. The resistance of the physiological system of the host to this stimulus.

The Effect of Continued Resistance upon the Extrachromosomal Stimulus

One of the striking things about the incidence of tumors in the dBF1 hybrids was that this generation had only one-half as much tumor as was shown by the dilute brown parental stock. This and the fact that those tumors which appeared did so at relatively late ages indicated that this hybrid physiology was more resistant to the action of the extrachromosomal stimulus than was the dilute brown physiology. The second filial generation of this cross produced even fewer tumors than the first. This indicated that continued resistance to the stimulus was breaking down its effectiveness. That this assumption had some factual basis is indicated by the behavior of the 8th backcross generation, in which the dBF1 hybrid females and their daughters were mated for 8 generations to C57 black males. No mammary tumors occurred in 180 virgin females of this generation. That this decline in

mammary cancer incidence is permanent is indicated by the results found in later generations. This cross is now in the 21st generation of inbreeding and no tumors have appeared during the last 13 generations.

the strength of the milk stimulus which the mice receive and the resistance of the physiological systems produced by these matings to various concentrations of the milk stimulus.

TABLE IV: GENERAL SUMMARY

Physiological system produced by:	Extrachromosomal stimulus	Resistance to stimulus	Ratio of mammary cancer
Homozygous dilute brown chromatin	Strong	Weak	100
Homozygous dilute brown chromatin derived from BdF1	Weak	Weak	0
Homozygous C57 black chromatin	Weak	Strong	1+
Homozygous black chromatin derived from dBF1	Weak	Strong	0
Homozygous black chromatin	Strong	Strong	10
Hybrid: dilute brown female × black male	Strong	Intermediate	52
Hybrid: black female dilute brown male fostered to dilute brown female	Strong	Intermediate	59
Hybrid: black female dilute brown male	Weak	Intermediate	2

THE EFFECT OF CONCENTRATING THE DILUTE BROWN CHROMATIN IN THE ABSENCE OF THE EXTRACHROMOSOMAL STIMULUS

If the 1st generation hybrids of the BdF1 cross, which have approximately 2 per cent mammary cancer, are backcrossed for 8 generations to dilute brown males, the amount of tumor in them does not increase. At the end of 12 generations of inbreeding, following the 8th backcross, dilute brown animals are obtained which develop no mammary tumors. Mammary tumors are not produced in the physiological system of the dilute browns in the absence of the extrachromosomal stimulus.

The observations recorded and discussed in this paper are summarized in Table IV.

SUMMARY AND CONCLUSIONS

In this paper an attempt is made to separate and evaluate the parts which chromosomal and extrachromosomal influences play in the development of mammary carcinoma in mice.

Data from nine experiments totaling 2,230 animals are offered as evidence that the ratios in which mammary tumors occur in inbred strains and experimental crosses may be reasonably explained upon the basis of

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Foster Nursing and Genetic Susceptibility for Tumors of the Breast in Mice*

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The object of this experiment was to determine whether foster nursing, with a decrease in the incidence of spontaneous carcinoma of the mammary gland, might produce any change in the genetic constitution of mice of an inbred strain. By genetic constitution is inferred the inherited susceptibility for the development of cancer of the breast.

All the mice used in the experiment were members of the A stock, a strain which has been inbred, brother-to-sister, since 1918. A female of the A strain (41st generation), No. 38432, and fostered by a female of the CBA or X stock gave rise to the line called the Ax strain (1, 4). All the females were continued as breeders.

The females of the Ax strain have a low incidence of mammary cancer while the females of the A (control) stock have a high incidence. The average age at death of the noncancerous females of the Ax strain was 8 months later than the average cancer age of the mice of the A stock (Table I).

Reciprocal matings were made between representatives of these two sublines. To distinguish between the hybrid generations, the maternal line is mentioned before the paternal stock in the designations; *e.g.*, high A♀ × low Ax♂ = AAxF1 and low Ax♀ × high A♂ = AxAF1. The mice of the second and following generations were usually produced by brother-to-sister matings.

The animals of the Ax strain available for the cross were members of the 47th to 53rd generations (7 to 12 generations since fostering of the ancestral female) and the mice of the A strain were from the 53rd to 58th generations. The controls were selected from the 54th to 61st generations (Table I). Observations on the incidences of tumors in the mice of the hybrid generations are given in Table I.

TABLE I: THE INCIDENCE OF MAMMARY CARCINOMA AND AVERAGE AGES OBSERVED IN BREEDING FEMALES OF THE A STOCK, FOSTERED FEMALES OF THE A STOCK (AX), AND HYBRIDS DERIVED FROM CROSSING MICE OF THE TWO LINES

Stock	Incidence in maternal stock	No.	Cancer incidence, per cent	Average age in months	
				Cancer	Noncancer
A(F54-F61)	High	299	98.0	9.4	11.2
Ax	Low	292	3.1	13.9	17.2
AxAF1	Low	107	0.0	...	17.4
AxAF2	Low	152	0.7	11.5	17.8
AAxF1	High	117	94.0	9.1	8.1
AAxF2	High	133	95.5	9.5	8.6
AAxF3	High	149	96.6	8.8	9.6
AAxF4	High	124 *	98.4	9.3	8.1
AAxF5	High	77 *	93.5	9.6	10.4
AAxF1-F5 (Total)		600	95.8	9.2	9.0

* Only depleted litters.

One mammary carcinoma was observed to develop in the hybrids descended from females of the fostered line. The noncancerous mice of the AxAF1 and F2 generations survived to an average age of 17.6 months; the single growth was observed at 11.5 months. The number of mice observed was 259.

The hybrids descended from maternal parents of the control strain had high incidences of mammary carcinoma. The results are complete for mice of the first 3 generations. In the 4th and 5th generations the observations include only litters in which all the mice have died. There has been no significant reduction in the incidence of breast tumors in any generation.

The total number of mice tabulated from the AAxF1 to AAxF5 generations was 600 and 95.8 per

* This investigation was aided by grants from the National Cancer Institute and The Jane Coffin Childs Memorial Fund for Medical Research.

cent of these developed tumors of the mammary glands. The average age at the time of recording the tumors in the controls was 9.4 months and in the hybrids 9.2 months. Twenty-five of the hybrids were noncancerous. Of this number only 8 lived to the average age for cancer.

Discussion

There may be two (or more) classes of spontaneous carcinoma of the mammary glands in mice, inherited and noninherited (2). Tumors of the breast may be said to be inherited or transmitted when the strain has a high incidence and the progeny of both cancerous and noncancerous mothers have high incidences characteristic of the strain.

For the development of inherited tumors in mice it has been assumed that at least three "influences" must be present and active (2). These may be:

(a) An inherited susceptibility for breast cancer.

(b) An active influence in the milk.

(c) An adequate hormonal stimulation of the mammary tissue.

Tumors developing in individuals having an inactive influence in the milk or which are not proven to be susceptible for mammary tumors generally are of the noninherited type. In such strains the incidence in the progeny of cancerous mothers is usually low and does not differ from the normal low incidence of the stock (3).

Breeding tests may be needed to determine the genetic constitution of mice of strains which have a low incidence of carcinoma of the breast. It is possible to have a strain in which the mice are susceptible for the development of breast tumors but which have an inactive influence in the milk. Should the influence in the milk become active *de novo* such a strain will change from low to high cancer (4). Also, a strain that is nonsusceptible for tumors of the breast but which has obtained an active influence by nursing will have a higher incidence than the unfostered mice of the strain. This incidence is low, however, when compared with the incidence observed in strains having a high incidence because of a combination of genetic factor and milk influence. On the other hand, fostered mice derived from the strain with a low incidence, having an active milk influence but usually noncancerous, may be used to nurse susceptible animals and a high incidence will result (3).

It is also possible to have a strain of mice developing few spontaneous tumors which have an active milk influence but do not possess the proper genetic constitution for the appearance of tumors. Only by mutational changes in genetic factors would this strain be expected to give a high incidence.

For the development of tumors induced by estrogenic hormones there is evidence that if the influence in the milk is active, the genetic susceptibility factor may not be required (8). Few tumors will develop in animals which have the proper genetic constitution but which have obtained an inactive influence when nursing (5, 7).

The data given above on the cross between cancerous females and males of a noncancerous line of the same strain showed that the incidence remained the same in the hybrids as in the controls. This demonstrates that foster nursing does not alter the inherited susceptibility for breast cancer in mice.

Whether or not we may consider the genetic constitution for the development of mammary cancer in mice as a quantitative or qualitative character is problematical. It may manifest itself by making the individual susceptible to the active influence in the milk usually obtained by nursing potentially cancerous females. This hypothesis may be reasonable if it is determined that the influence in the milk is a virus (6) and, to date, no evidence to the contrary has been observed. It is not claimed, however, that the hypothesis provides proof of the virus-nature of the influence.

Conclusion

In mice foster nursing does not influence the genetic susceptibility of an individual for the development of spontaneous mammary carcinoma.

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Abnormalities of Breeding Behavior in Rats of the Albany (A-S) Strain*

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In previous publications we have described abnormalities of reproduction, as well as of growth, in a colony of rats (Albany or A-S strain), in which spontaneous mammary tumors have appeared with a frequency unusual for this species (1, 2, 7). This colony, now numbering about 2,400 rats, of which approximately 1,400 are females, represents descendants of 8 females from the original group of 51 described in the first report (1). The incidence of tumors varies among the sublines, but for the colony as a whole it is at present 24 per cent among the females reaching the age of approximately 15 months.

From the beginning, great difficulties have been encountered with the development of high tumor lines because of the low fertility which is manifested by the rats of the A-S strain. Investigations into the causes of the high incidence of sterility revealed that, when compared with rats of a normal strain (Vanderbilt or V-S strain), a large proportion of these animals exhibit atypical estrous cycles associated with an ovarian dysfunction which results in frequent or repeated failure of ovulation (7). Furthermore, among the females that do mate successfully, a high frequency of spontaneous fetal resorptions occurs which reduces materially the numbers of young delivered (2). In the present report we wish to describe another abnormality in the breeding behavior of the A-S rats, namely, a strikingly high rate of infanticide [1] or cannibalism.

In general, all lines of the A-S strain have been bred by brother-to-sister matings, but it has been necessary to breed some females with their fathers, or even with their uncles or cousins, in order to continue certain sublines. Such close inbreeding was not followed in the V-S colony, in which no attempt was made to develop sublines.

The first matings are made when the males and females are from 90 to 120 days of age. When the placental sign is first observed in the vaginal smear, the presence of a pregnancy is confirmed by palpation of the abdomen for fetal sites. The pregnant females are then isolated. Litters are weaned at 25 days of age, and the females are mated again directly after the young have been separated. The rats of both colonies are fed a standard, commercially prepared food, Wayne Dog Food Blox,[2] supplemented with lettuce once or twice a week.

In the course of our efforts to develop a strain of rats which would yield a high incidence of mammary tumors, it became apparent that a high rate of infanticide or cannibalism existed among the breeding females of the A-S strain. This is clearly indicated in Table I, which gives a composite summary of the breeding histories of A-S females which eventually developed tumors, those which have not developed tumors up to the present time, and V-S females which do not have tumors and were used as controls.

In order to determine to what degree cannibalism was related to the age of the breeding female, as well as to the presence of tumors, the data were analyzed from two points of view; namely, for the period up to the average tumor-bearing age for the A-S colony, which is approximately 15 months, and for the subsequent period represented by an age range of 16 to 25 months.

It is at once apparent from the data in Table I that the proportion of litters killed by the A-S females even in the earlier breeding period is strikingly high, as compared with the degree of cannibalism manifested by the control V-S group, which, on the basis of incidental statements such as that by Wiesner and Sheard (6) may be taken as the order of magnitude which exists among colonies in general. Without emphasizing the significance of the differences which are shown, it is nevertheless interesting that even in the younger age group the highest rate of cannibalism in rats of the A-S strain was manifested by those animals which later developed mammary tumors. It is hazardous to evaluate on a percentage basis the marked increase in infanticide which occurs in the later age period because of the much smaller total number of litters delivered at the older age period. In view of the continued low rate of cannibalism in the V-S strain, even in the older rats, it does not

* This investigation was aided by a grant from The International Cancer Research Foundation.

[1] In this paper infanticide is used to mean not only the killing but also the eating of the young by the mother.

[2] Manufactured by the Allied Mills, Inc., Chicago, Ill.

appear likely that such behavior tends to develop as a result of repeated pregnancies or that it is intimately related to aging *per se*.

Several interesting ancillary facts become evident from a study of Table I. Fertility of the A-S animals, for example, is generally poor. Those animals which eventually became tumor-bearers showed even in their early breeding periods the lowest percentage of pregnancies, only half of the matings in this group resulting in pregnancy. In the nontumor-bearing group only three-quarters of the matings eventuated in pregnancies. The animals of the control colony, on the other hand, although their number was considerably smaller, showed a much higher degree of fertility.

When comparison is made of the data for the periods before and after the tumor bearing age, it is seen that all groups showed a decrease in fertility, the order being approximately one-half for the tumor-bearers, one-third for the nontumor females of the A-S strain, and one-fifth for the control V-S animals. On the basis of the evidence obtained from the V-S females, it is apparent that age is partly responsible for the greater frequency of sterility in the older A-S females. Nevertheless, the fact that the tumor-bearing group showed a definitely greater reduction of successful matings than the tumor-free group of the same strain suggested that the development of the tumor and a high order of sterility may be associated phenomena.

It is furthermore noted that the number of pregnancies which terminated in complete resorption of the fetuses was high in the A-S colony. This phenomenon has been previously reported (2), but the present observations are based upon the study of more than twice the number of pregnancies comprising the original study and are therefore more significant.

In considering further the problem of cannibalism in the A-S colony, the data at hand were studied with the view of answering the following questions: Is there a difference in the extent to which death among the litters is attributable to cannibalism, and if so, which litters, the early or the late ones, are destroyed most frequently? Do the mothers, once they manifest the cannibalistic tendency, eat their litters repeatedly? How soon after delivery do the cannibalistic mothers eat their litters?

Table II gives the distribution of a total number of 750 litters eaten. The calculations of percentages give reliable information only for the first 5 or 6 litters as the samples (total number of litters delivered) become progressively smaller to the degree where critical evaluation is impossible. It can be concluded that the rate of infanticide is, within the laws of chance, of approximately equal magnitude for the first 4 litters and that there may be a tendency for this habit to become accentuated for the later litters.

In Table III it can be seen that it was not possible to predict that the cannibalistic tendency would be manifested for the subsequent litter, as it can be seen that some of the rats ate their litters haphazardly. It is an interesting fact that one of the animals which killed 9 litters was a tumor-bearing female which had 10 litters in all, and that the only litter which the animal weaned was the 7th. Such inconstant behavior made it impossible to predict whether or not it was profitable to breed such animals, and all were continued to be bred in the hope of obtaining some litters,

TABLE I: COMPARISON OF THE EFFECT OF TUMOR DEVELOPMENT AND OF AGE ON REPRODUCTIVE BEHAVIOR

Group	Number of rats	Number of matings	Pregnancies		Resorptions		Number of live litters delivered	Litters killed		Litters weaned	
			No.	Per cent	No.	Per cent		No.	Per cent	No.	Per cent
BEFORE AVERAGE TUMOR BEARING AGE *											
A-S tumor-bearers	182	745	371	50	71	19	299	109	37	190	63
A-S nontumor-bearers	189	889	645	73	95	15	550	146	27	404	73
V-S controls	63	268	261	97	8	3	252	12	5	240	95
AFTER AVERAGE TUMOR BEARING AGE †											
A-S tumor-bearers	161	238	59	25	18	31	41	28	68	13	32
A-S nontumor-bearers	189	313	158	50	48	30	108	50	46	58	54
V-S controls	23	44	34	77	3	9	31	2	6	29	94

* The average age of appearance of mammary tumor in our colony was 15.4 months. The age range of the breeding females in this period was from 5 to 15 months.

† The breeding period of these animals ranged from 16 to 25 months.

TABLE II: THE DEGREE OF MORTALITY AMONG THE LITTERS BECAUSE OF INFANTICIDE

Litter number	1	2	3	4	5	6	7	8	9	10	11	12	Total
Number of litters delivered	893	618	409	261	150	84	46	25	10	3	1	1	2,501
Number of litters killed	285	137	120	75	60	32	20	12	5	2	1	1	750
Percentage	31.9	22.2	29.3	28.7	40.0	38.1	43.5	48.0	50.0	..	..	..	29.9

particularly in those lines which showed a high incidence of tumor development.

Table IV reveals that 45 per cent of the litters eaten were consumed during the day of delivery, but that almost as many were lost by cannibalism during the rest of the first week post-partum, and that 15 per cent of the animals eventually killed their young after nursing them for a week or more.

Discussion

Although a certain degree of cannibalism has been observed among rats by many investigators, only a few scattered reports can be found which mention this phenomenon. Slonaker and Card (5) described in some detail the increase in cannibalism in rats deprived of food from animal sources, but these studies were made before synthetic diets were widely used. The paucity of reports on the possible causes of cannibalism may be the result of the relatively low incidence of this abnormality among rats. Thus rats of the A-S strain are ideal animals for studies on cannibalism, since the rate is unusually high, and experiments are being planned to determine to what extent nutrition plays a causative role.

Table III: The Frequency with Which the Mothers Ate their Litters

Number of litters eaten	Number of rats which ate litters	
	Consecutively	Haphazardly
2	89	16
3	36	4
4	9	2
5	9	4
6	2	2
7	1	0
8	0	1
9	1	1

Table IV: The Frequency Distribution of the Number of Litters Eaten Correlated with the Days Following Delivery

Period after delivery, days	Number of litters eaten	Percentage
0-1	340	45
2-7	301	40
8-14	96	13
15-25	13	2

It may also be that a hormonal deficiency is in part responsible for cannibalism. Because of the evidence that rats of the A-S strain have a dysfunction of the anterior lobe of the pituitary gland (7, 8), preliminary studies on the administration of prolactin were made in an attempt to determine whether or not the lactogenic hormone may be responsible for maternal behavior, as reported by Riddle, Lahr, and Bates (4). The results have proved disappointing, but this is not surprising in view of the observations made by Leblond and Nelson (3) that the anterior lobe is not essential for the manifestation of parental instinct, since it is present in animals even after hypophysectomy.

It has been consistently observed throughout all of the studies of the A-S rats that, although the colony as a whole, when compared with a normal strain, shows gross abnormalities in reproductive physiology, there is a considerable tendency for the abnormality to be present to a greater degree in the tumor-bearers. Thus it has been observed that while the A-S strain as a whole has an abnormal frequency of epithelial or cornified cells in the vaginal smears when compared with a normal strain, tumor-bearing animals tend to show this abnormality to a greater extent than do those which are tumor-free (7). In the same manner, while the number of pregnancies for the A-S strain is significantly lower than that of a normal colony, pregnancies occur least frequently in the tumor-bearers (7). It is also apparent from the present studies that those animals which eventually develop tumors have, even in their early breeding periods, a lower percentage of pregnancies than do the animals which remain free of tumors. Finally, it has been observed that although cannibalism is frequent in both tumor-bearing and nontumor-bearing rats, those with tumors show this tendency to a greater degree even in the younger age period before the tumors actually develop. There is thus a consistency in quantitative differences between animals of the A-S strain which are destined to develop tumors and those which are not. This trend toward exaggeration of abnormality in the tumor-bearing group appears to us to be more than accidental and is probably related to tumor development in a way that is as yet obscure.

Summary

In the present report an abnormality in the breeding behavior of the A-S rats is described, namely, a strikingly high rate of infanticide. The data of the breeding histories of the A-S females with and without tumors reveal that both groups of A-S rats have an unusual tendency to kill their young before and after the tumor age, but that the tumor-bearers show this tendency to a greater degree. Comparison with a normal breeding colony suggests that the cannibalistic habit does not appear to be associated with aging *per se*.

The extent of cannibalism in the A-S strain is, even for the first litter, similar in magnitude to the rate taken for the group as a whole, indicating that this habit is not merely the result of an eventual deterioration of the maternal behavior pattern. The killing habit, once established, does not always persist but

may be manifested erratically for only a few of the total number of litters cast by the female. In a great many instances the young are eaten on the day of delivery. The possible mechanisms involved in this irregularity of breeding behavior are discussed.

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Action of Yeast Extract on Transplanted and Spontaneous Malignant Tumors in Mice*

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In previous communications (2-5) we have reported that injections of certain extracts of spleen and of yeast have been followed by regression of spontaneous and transplanted mammary adenocarcinomas in several strains of mice. The purpose of this report is to present the results obtained by the intravenous injection of various fractions of extract of yeast into mice bearing spontaneous and transplanted adenocarcinomas.

Chemical Studies

As a test for the efficiency of the various fractions obtained from the yeast extract we employed the therapeutic action of the material when given intravenously to mice bearing spontaneous breast adenocarcinomas. The yeast extract which was used as the starting material had rendered 25 to 30 per cent complete regressions among the treated animals. It was believed that a fraction could be accepted as satisfactory if it yielded, in a smaller dose, the same results as the original yeast extract. In order to determine the potency of a fraction, about ten animals were taken for a preliminary experiment. If complete regression occurred in some of these mice, the experiment was repeated with 25 to 30 animals in order to ascertain whether the percentage healed with the fraction differed appreciably from that obtained with the original yeast extracts.

The yeast extracts, used for the fractionation, were always prepared as described in earlier communications (2, 3). Twenty-five different lots of fresh brewer's yeast of about 2.5 kg. each have been used in the last two years and each of them has been found to contain material acting upon tumors. No loss of potency was observed on storage of the extracts under sterile conditions over a period of several months.[1]

In view of the fact that yeast provides a very rich source of the vitamins of the B complex, we have been interested in the possible role of some of these vitamins as specific or contributing factors in the regression of tumors. Pyridoxin, pantothenic acid, and biotin, when injected singly, did not influence the growth of spontaneous adenocarcinomas. In contrast to the observations on butter yellow liver cancer (1) and our own experiments on prevention of transplanted carcinoma 2163 (4) the combination of riboflavin or pantothenic acid with yeast did not change the results obtained with yeast alone in the treatment of spontaneous breast tumors. The vitamin assay of one of our active fractions (No. 3, Table I) showed that, in the dosage given, the yeast fraction contains only negligible amounts of pantothenic acid, riboflavin or biotin, and from chemical considerations it can be concluded that the same is likely to hold true for thiamin and nicotinic acid.[2] Therefore, it seems reasonable to assume that these members of the B group are not involved in the phenomenon studied.

The fact that the active principle passes through cellophane membranes suggests that it is not of protein nature, as reported in a previous paper (3). It can be precipitated from the dialysate by high concentration of ethanol. Thirty-seven mice have since been treated with such a fraction, 9 of them successfully (No. 1, Table I). The nondialyzable part and the alcoholic filtrate of the dialysate were inactive. By alcohol-precipitation most of the toxic fraction causing shock is eliminated and passed into the filtrate, whereas the toxicity of the active precipitate is markedly reduced as compared to the total yeast extract.

In order to replace the removal of proteins by a less time-consuming procedure than dialysis, the yeast extract was precipitated with lead acetate. The resulting precipitate and protein-free filtrate were tested in animals and the activity found to have passed into

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[1] Centrifugation and washing of the yeast cells as described (3) was found to be useful, as the supernatant and the washings did not contain appreciable amounts of active material but large quantities of inactive solids.

[2] 1 gm. of fraction No. 3 contains 10γ of riboflavin, 25γ of pantothenic acid, less than 0.01γ of biotin, and less than 10γ of pyridoxin.

the filtrate. Among 19 animals treated with the lead filtrate fraction, 5 complete regressions occurred (No. 4, Table I). It appeared, however, that some potency had been lost in this procedure. If the lead precipitation is followed by treatment with silver nitrate at pH 5, the activity is again found in the filtrate, whereas the material (primarily purine substances) precipitated at this pH by silver is ineffective. Sixteen animals received the silver filtrate fraction; in 4 of these the treatment was successful (No. 6, Table I). After removal of inactive substances by salts of lead and silver, a fraction was prepared which contained the barium salts insoluble in 60 per cent ethanol. The potency of this preparation has been tested so far on 11 animals only; the tumors regressed completely in 4 of these mice (No. 8, Table I). Furthermore it was possible to precipitate an active fraction from the silver filtrate fraction by means of adsorption gave negative results in the biological test. Most of the activity is absorbed by norite from neutral solutions. The elution of the potent material from the norite adsorbate by water-methanol-pyridine mixtures has not been successful as yet.

From the experiments described it is evident that the active principle is readily water-soluble; all active fractions obtained up to the present could be precipitated from aqueous solutions by high concentrations of ethanol.

The procedures outlined involved changes of the hydrogen ion concentration from pH 1.5 to 8.5. Under these conditions no loss of potency could be detected. However, activity was destroyed by treatment of an active alcohol precipitate with N/2 hydrochloric acid, for 3 hours at room temperature. Nine animals received the acid treated material; no regressions occurred (No. 11, Table I). Nitrous acid was

TABLE I: SUMMARY OF TESTS OF FRACTIONS OF YEAST EXTRACT

Extract No.	Preparation	Dose in mgm.	Per cent of solids compared with yeast extract	Total number of animals	Complete * regressions
1.	Ethanol precipitate after dialysis	3	28	37	9
2.	Lead filtrate from No. 2	3	22	27	9
3.	Silver filtrate from No. 3	2	11	23	7
4.	Lead filtrate from No. 1 (not dialyzed)	4	25	19	5
5.	Lead precipitate from No. 1	4	37	7	0
6.	Silver filtrate from No. 5	1.5 1.5	16	16 †	5
7.	Silver precipitate from No. 5	1	11	8	0
8.	Ethanol-insoluble barium salts from No. 7	0.9	3.2	11	4
9.	Phosphotungstate from No. 7	0.7	2.5	9	3
10.	No. 2 after nitrous acid treatment	7.5	..	7	2
11.	No. 2 after treatment with N/2 HCl	4.5	..	9	0

* Temporary regressions with subsequent recurrences are not included in these statistics.
† 8 mice received 1.5 mgm., and 8 received 4.5 mgm. in single doses.

phosphotungstic acid. In a preliminary experiment the phosphotungstates were administered to 9 animals with the result that the tumors regressed completely in 3 of the mice (No. 9, Table I).

As the direct precipitation of the yeast extract with lead acetate seemed to involve a certain loss of activity, the lead treatment was repeated with the dialysate of the yeast extract. The resulting lead filtrate fraction was of satisfactory biological activity. Of the 27 animals used to test this fraction, 9 were treated successfully (No. 2, Table I). As in the case of the nondialyzed yeast extract, activity did not appear in the silver nitrate precipitate at pH 5; it was present and caused complete regression in 7 mice among 23 (No. 3, Table I).

Adsorption experiments carried out so far have given the following results: Permutit at pH 6 does not retain the active principle; the potency can be recovered from the filtrate. When treated with Fuller's earth at pH 4.5 the active principle is removed from the solution to a large extent; a filtrate from such an without detectable influence upon the potency of the extracts; of 7 animals taken to test the nitrous acid treated material, complete tumor regression was observed in 2 of them (No. 10, Table I).

The method used to prepare the extracts from the yeast cells illustrates that the active principle is rather thermostable around pH 6. However, no information is available as to the rate of destruction at elevated temperatures. Therefore in all fractionation procedures the temperature was kept below 40° C. and pH between 5 and 6 whenever possible.

Autolysis of the yeast cells previous to the extraction yielded an ineffective extract, indicating that enzymatic destruction had taken place.

In our original yeast preparation the margin between effective and lethal dose had been rather narrow (curative dose, 5 mgm., lethal dose, 10 mgm.), and shock always occurred in the mice following the injection. Intravenous injection into normal anesthetized dogs or cats of the yeast extract caused a marked fall of blood pressure. From our subsequent fractions

the shock-producing substances are largely removed; *e.g.*, fraction No. 3, Table I (effective dose 2 mgm.) could be injected intravenously to mice in amounts up to 50 mgm. without producing more than a slight reaction.

These results indicate that at least a part of the active principle present in the original yeast extracts may be obtained in the form of a fraction with the properties of preparation of No. 9, Table I. Since the present testing method does not yet present a rigid quantitative evaluation of the activity with respect to causing regression of tumors, no attempt has been made to appraise the yield of the active principle obtained in the various stages of preparation. The figures in the fourth column of Table I, indicating the amounts of total solids of original extracts corresponding to the total solids of the various fractions, convey a general idea of the degree of purification.

We wish to emphasize that occasionally fractions have been encountered in which very little or no activity could be demonstrated although they had been prepared by the same procedures as fractions found to be of good potency; *e.g.*, of 2 fractions prepared in the same manner as fraction No. 1, Table I, one was devoid of any activity when tested on 20 animals whereas the other preparation showed only 4 temporary regressions in a group of 28 animals. Similarly, with a fraction corresponding to No. 4, Table I, treatment was successful only with 3 animals among 21. Since until recently only small lots of yeast were worked up at a time we do not know whether these results are due to variations in the starting material.

Intravenous Treatment of Spontaneous Mammary Carcinomas in Mice with Extract of Yeast

In a previous paper (3) we reported results of treatment with yeast extract on spontaneous mammary carcinomas arising in strain A mice from the Roscoe B. Jackson Memorial Laboratory.

During the last year we have tested the action of yeast extract on three other malignant tumors. The following mouse tumors were studied:

a). The Rockland Farm spontaneous breast carcinoma. This adenocarcinoma is not an inbred tumor strain, but occurs in mice of the older age group. We have worked intensively with this tumor. The percentage of complete disappearance following intravenous injections of yeast extract is 30 per cent (exactly the same as in the strain A from the Jackson Memorial Laboratory).

b). The spontaneous carcinoma in the French strain R III mice (Dobrovolskaia-Zavadskaia). The results of treatment among 12 animals are shown in Fig. 1. We treated a total of 20 animals. Seven animals among these 20 animals (or 9 tumors among 22) showed complete disappearance of the tumors. Three additional tumors among these 20 nonbiopsied animals were healed clinically, but showed microscopically remains of tumor cells at post-mortem examination. These tumors were not subjected to a biopsy, in order to see whether identical results could be obtained in biopsied, and nonbiopsied malignant tumors. Results were identical in biopsied and nonbiopsied tumors of this strain (See Table II).

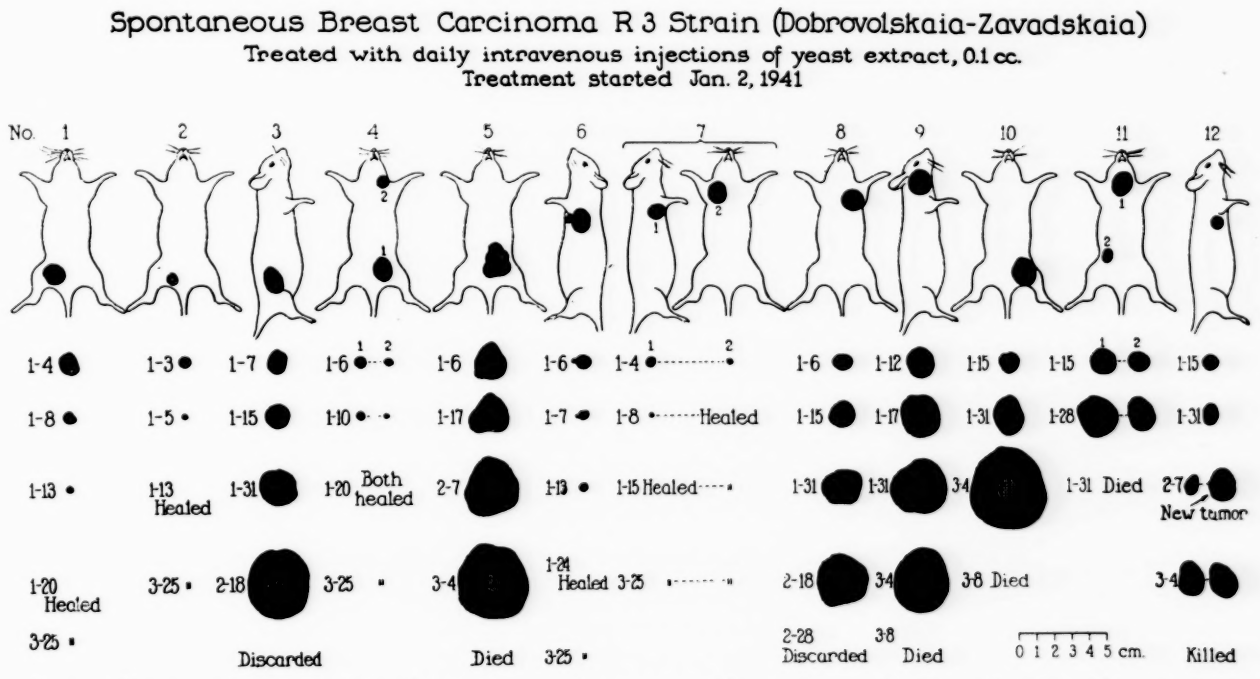


Fig. 1.— Diagrammatic summary of results of treatment of spontaneous adenocarcinoma of the breast in mice with yeast extract.

c). The third tumor is not a spontaneous, but a transplanted tumor (transplanted carcinoma 2163 in the R III strain). This tumor is a mammary adenocarcinoma which in this laboratory was found to be transplantable in 95 to 100 per cent of mice of this strain, the strain in which it arose. In our experience this carcinoma has proved very resistant to treatment.

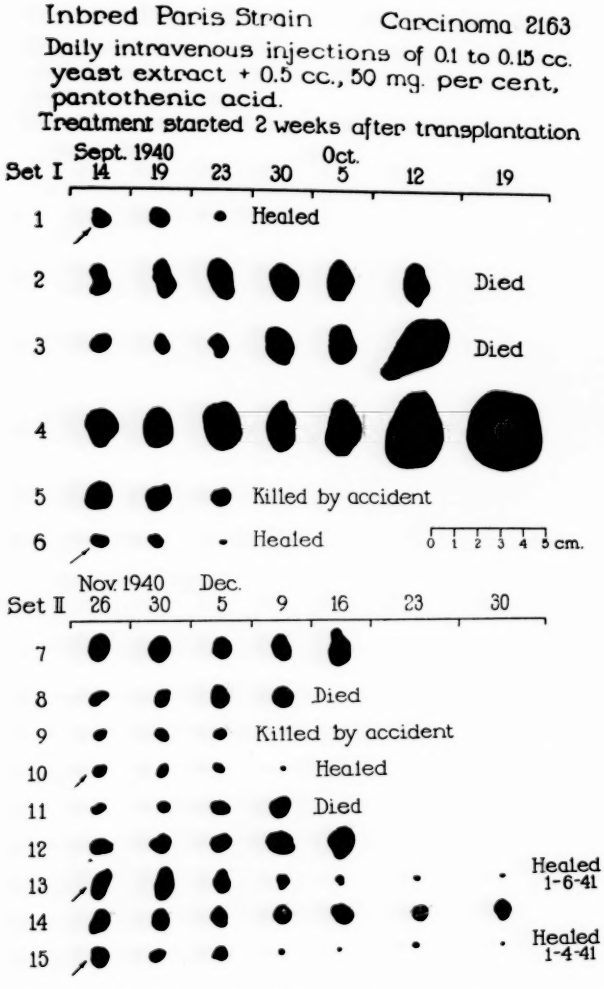


Fig. 2.—Diagrammatic summary of results of treatment of transplanted carcinoma 2163 in mice with yeast extract and pantothenic acid.

We present our results in Fig. 2. Fig. 3 shows the growth among the controls. Five among 15 treated tumors disappeared whereas in untreated mice these transplanted tumors may grow to relatively enormous size.

Table II: Results of Treatment of Spontaneous Adenocarcinoma of the Breast in Strain R III Mice, with and without Biopsy

	Number of animals treated	Number healed	Number not healed
After biopsy	20	7	13
Without biopsy	20	7	13

So far we have treated successfully different transplanted and spontaneous malignant tumors in white mice. We have not been able to obtain similar results in brown or black mice (for instance C3H). The reason may be purely technical. It often requires 30 to 40 intravenous injections into the tail veins of the mouse before the tumors disappear. Intravenous therapy is very difficult in dark mice, as the veins are

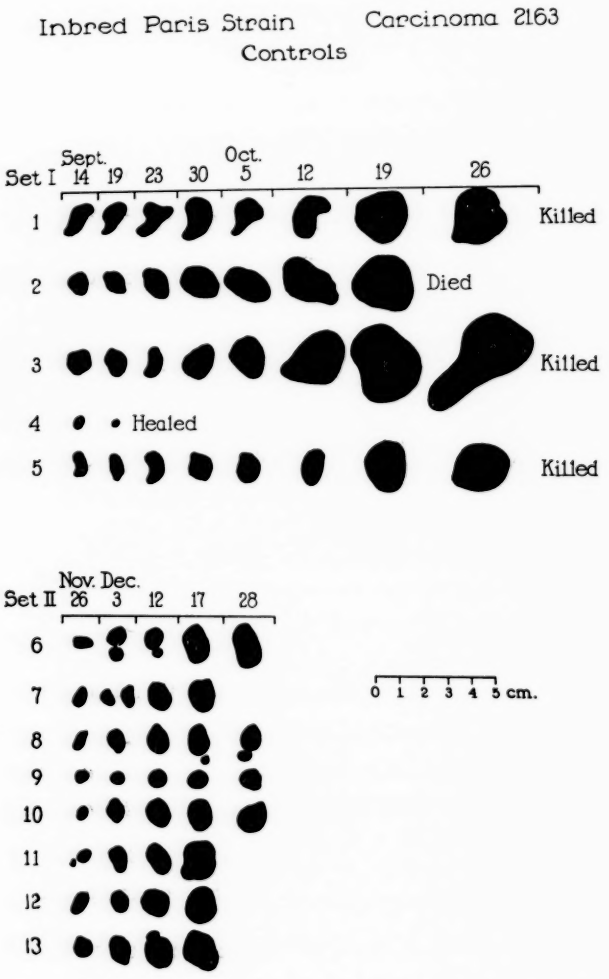


Fig. 3.—Diagrammatic summary of growth of transplanted carcinoma 2163 in untreated mice.

not clearly visible and become blocked after a few injections.

As a rule we have not been able to influence large spontaneous tumors though some successful treatment of larger tumors is shown in Fig. 4. Sometimes, however, small tumors are more resistant than large tumors.

The time which elapses between the first treatment and the date of the disappearance of the tumors very often depends on the size of the tumors. As a rule small tumors respond to the yeast extract quicker than large tumors.

Biopsy without treatment fails to cause complete regression of these tumors. In a recent paper (5) we reported our results with simple biopsy in 82 strain A mice from the Jackson Memorial Laboratory bearing spontaneous adenocarcinomas of the breast. In none of these animals did the tumors disappear as a result of the biopsy.

In a previous communication (3) we have reported that tumors recur in about 25 per cent of the apparently healed animals. These recurrences are usually noted 6 to 8 months after the disappearance of the original tumors. These recurrences (or new tumors in the approximate location of the original tumors) are absolutely resistant against yeast extract therapy.

Table III shows the time elapsed between the disappearance of the tumors and the death of the animals. As these tumors usually appear when the animals are about 15 months old and as the life expectancy of a mouse is about 2 years, the vast majority of the animals died a few months after the disappearance of the tumors. Yet we had occasion to observe mice up to 15 months after the healing of the carcinoma without a recurrence or appearance of a new tumor.

Table IV shows an additional 20 animals still living, and apparently healed.

Among the 70 animals healed by spleen or yeast extracts 55 belonged to strain A Jackson Memorial Laboratory and 15 came from the Rockland Farms.

We have not continued active work with spleen extracts because we found that the preparation and standardization of the yeast extract was much simpler

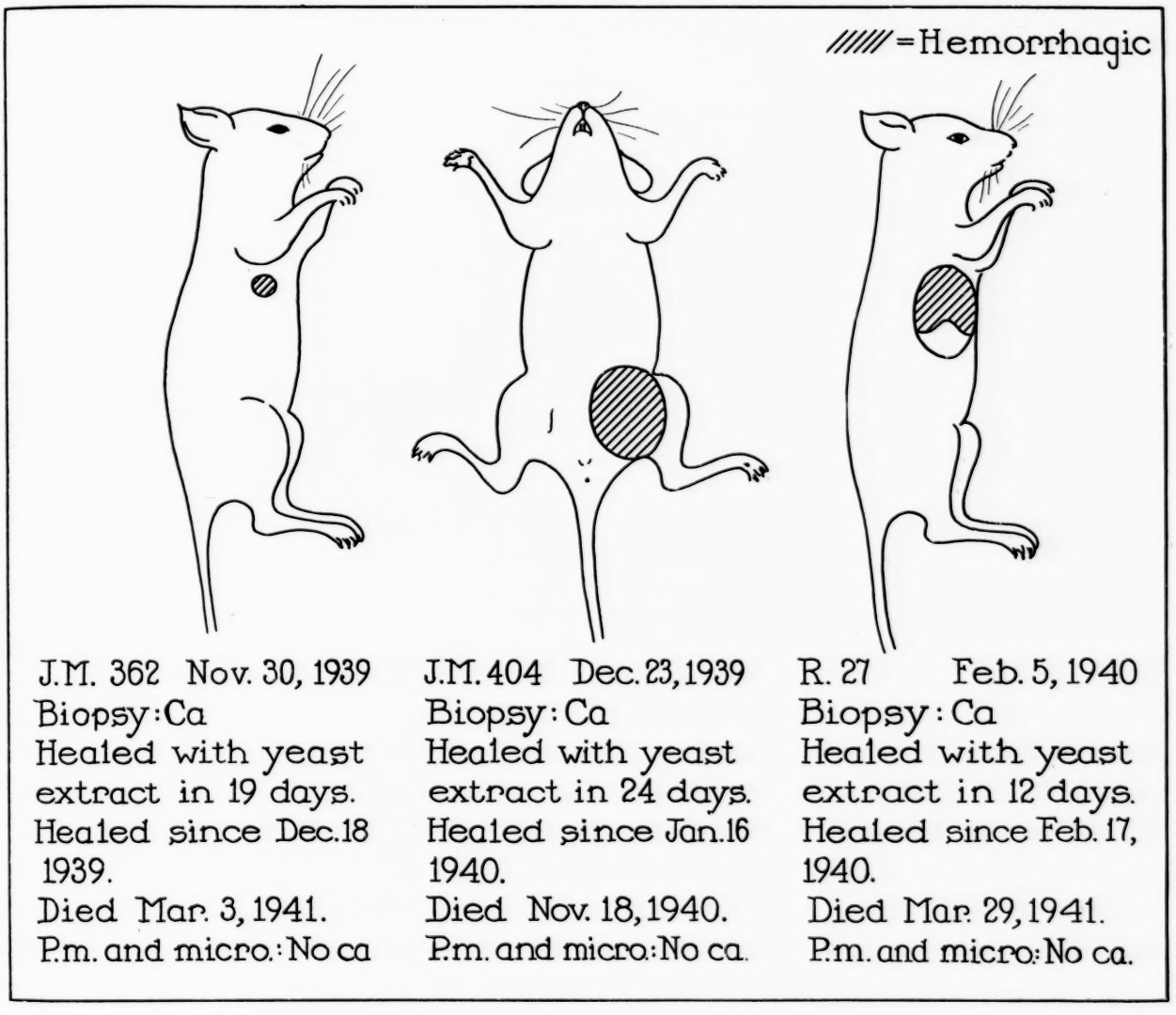


FIG. 4.—Diagram of tumors in mice healed following injections of yeast extract.

than that of the spleen extracts. For this reason we have confined our efforts during the last year to experimental work with yeast extract.

TABLE III: TREATMENT WITH SPLEEN EXTRACT AND YEAST EXTRACT; TIME ELAPSED BETWEEN DISAPPEARANCE OF TUMORS AND DEATH

Spleen extract		Yeast extract	
Time elapsed Months	No. of animals	Time elapsed Months	No. of animals
1	4	<1	4
3	1	1	4
4	2	1½	5
5	1	2	3
6	1	3	3
7	1	3½	1
8	1	4	4
12	2	5	1
14	2	6	2
15	1	7	1
(Missing after 2 months)	1	9	1
	..	10	3
	..	11	1
	..	12	2
Total....	17	Total....	33

TABLE IV: TREATMENT WITH SPLEEN EXTRACT; TIME ELAPSED SINCE DISAPPEARANCE OF TUMORS AND JUNE 1, 1941

Time elapsed Months	No. of animals
<2	1
2	8
3	6
4	1
5	3
6	1
Total....	20

HISTOLOGICAL STUDIES OF THE EFFECT OF YEAST EXTRACT ON SPONTANEOUS MALIGNANT TUMORS

We feel justified in calling an animal "healed" when complete macroscopic and microscopic postmortem examination of a clinically "healed" mouse fails to reveal any tumor tissue or tumor cells. However, not infrequently we find small nests of markedly changed cells suggesting remnants of former tumor cells. When such cell groups are present it may be impossible to decide whether they represent viable tumor cells. In view of the difficulties of the interpretation of such findings we have classified these animals as "nonhealed" though it is possible that these cells have lost completely their malignant character.

On the other hand, the late recurrences which we have noted in apparently healed tumor mice may originate from such temporarily dormant nests of malignant cells.

The proper interpretation of the regressive changes in the tumors of the treated animals is difficult, because similar changes, though less extensive, may be observed in untreated tumors. However, in successfully treated animals the whole tumor undergoes marked changes in a relatively short time, whereas in the controls these changes occur more slowly and appear more localized.

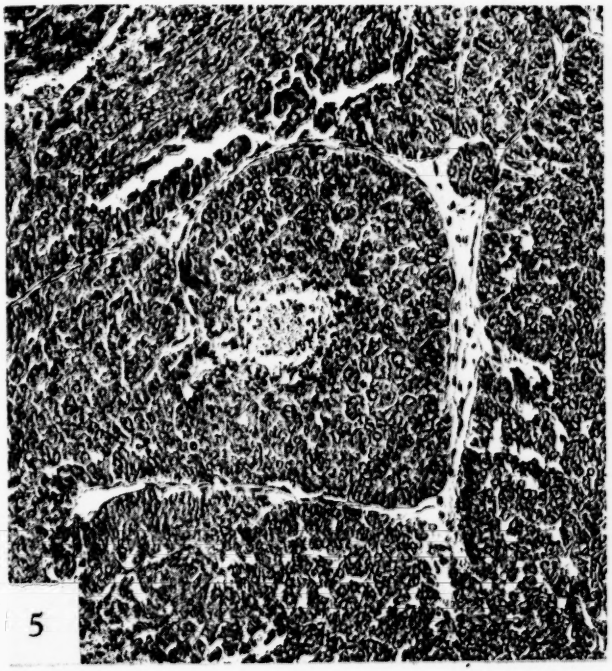
A comparison of the original specimen obtained at biopsy with an early stage of treated and influenced tumors shows the marked changes in the histological appearance of the tumor even after a few intravenous injections of yeast extract. The changes in the histological picture of these tumors at different periods during treatment with yeast extract will be presented in detail in a separate paper. Suffice it to state here that the tumor cells gradually diminish in size and the nuclei become pyknotic, the cells simulating lymphoid elements. Coincident with this alteration in the character of the cells, the tumor gradually shrinks until it may disappear completely, or may be changed to a small fibrous node. At present we have no definite histological criterion which would enable us to predict the ultimate complete disappearance of a tumor under treatment. We must keep in mind that at any time during the course of the treatment such tumors, in spite of temporary shrinkage, may suddenly start to grow again.

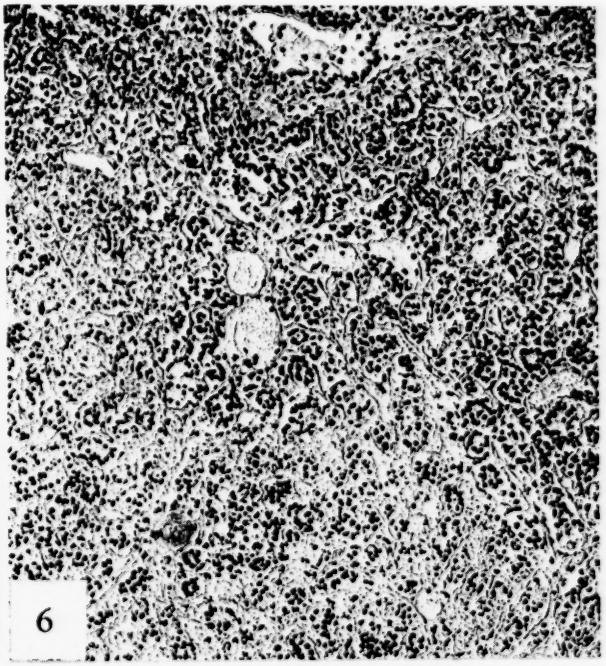
Fig. 5 shows a section of the specimen removed at biopsy of a strain A Jackson Memorial Laboratory mouse. This biopsy was taken before the treatment was started. It presents a typical adenocarcinoma. The same tumor, completely changed after seven intravenous injections of yeast extract, is shown in Fig. 6. Instead of well preserved carcinoma cells, we find cells in different stages of necrosis. Shrinkage and lymphoid appearance of the tumor cells is apparent.

Fig. 7 presents a biopsy specimen from a spontaneous adenocarcinoma of the breast in a Swiss mouse of the Rockland Farms strain. Fig. 8 shows the appearance of the same tumor after 43 intravenous injections of yeast extract. Marked changes in this tumor are noted. It presents a fibrous node with partial hyalinization. Small cords of lymphoid elements are imbedded in these fibrous areas. Some of these lymphoid elements probably represent changed tumor cells.

SUMMARY

In testing the action of fractions of yeast extract on the regression of spontaneous adenocarcinoma of the mouse, the following results were obtained. The active principle is water-soluble, and comparatively thermostable at neutral pH. It is not protein in nature, not affected by nitrous acid, nor precipitated by high





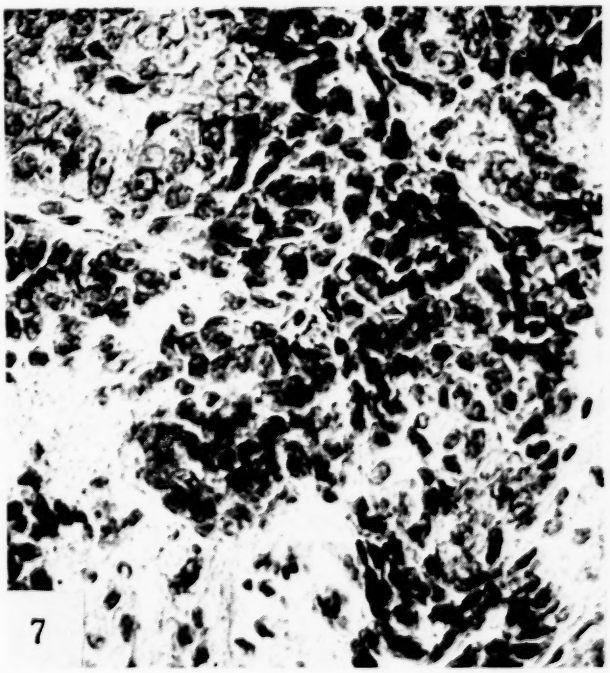


FIG. 5.—Photomicrograph of section of spontaneous mammary adenocarcinoma No. 314, Roscoe B. Jackson Memorial Laboratory. Specimen removed at biopsy on a strain A mouse. Mag. × 160.

FIG. 6.—Photomicrograph of the same tumor (Fig. 5) after 7 intravenous injections of yeast extract, showing necrosis of cells. Mag. × 160.

FIG. 7.—Photomicrograph of section of a spontaneous adenocarcinoma of the breast, N.o. 235, in a Swiss mouse of the Rockland Farms strain. Specimen removed at biopsy. Mag. × 200.

FIG. 8.—Photomicrograph of the same tumor (Fig. 7) after 43 intravenous injections of yeast extract, showing disappearance and lymphoid change in tumor cells, lymphocytic infiltration, fibrosis, and hyalinization. Mag. × 160.

concentration of ethanol. The active material is precipitated by lead acetate and silver nitrate. Active preparations can be obtained by precipitation with barium and ethanol, and also by phosphotungstic acid. The active material is adsorbed by Fuller's earth and by norite but not by permutit. It has not been possible to remove it by elution. None of the known vitamins of the B group appears to be responsible for the activity.

Results with yeast extract on four different malignant tumors in mice are reported. Three were spontaneous mammary adenocarcinomas in mice of the following strains: 1. strain A, Jackson Memorial Laboratory; 2. Rockland Farms strain; 3. strain R III. One tumor was the highly malignant transplanted carcinoma 2163 in the R III strain. With all these tumors 30 per cent complete disappearance was produced.

In the spontaneous carcinoma (R III strain) results obtained in biopsied and nonbiopsied tumors were found to be identical. As a rule small tumors respond to the yeast extract more quickly than large tumors. Recurrences are noted in about 25 per cent of the apparently healed animals, usually after 6 to 8 months.

Fifty spontaneous mammary carcinomas are presented. Biopsy performed before treatment was started had established the diagnosis of carcinoma. Intravenous treatment either with spleen or yeast extract was given. The tumors had disappeared completely for 1 to 12 months when the animals died. Careful post-mortem and microscopical examination failed to show any evidence of remaining tumor cells. Twenty animals are still living and apparently healed. Among the 70 healed animals, 50 belonged to the strain A Jackson Memorial Laboratory and 20 to the Rockland Farms strain.

The interpretation of the regressive changes in the tumors of the treated animals is difficult, as similar changes, though not as extensive, may be observed in untreated tumors. However, in successfully treated animals the whole tumor undergoes marked changes in a relatively short time, whereas in the controls these changes occur more slowly. Remnants of unchanged malignant tumor tissue are always present in the controls. Histological pictures of marked changes in treated tumors (as compared with the original biopsy specimen) after 7 and 43 intravenous injections respectively are presented.

The authors express appreciation for the valuable cooperation and help in the chemical studies received from Messrs. Merck & Co., Rahway, N. J.

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The Mechanism of Carcinogenesis

A Study of the Significance of Cocarcinogenic Action and Related Phenomena

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In a previous communication (5), the author has shown that when the effective carcinogenic potency of 3,4-benzpyrene for the mouse's skin was reduced by application of the hydrocarbon in a dilution of 0.05 per cent, the low tumor yield (0 to 6 per cent at the 20th week) was increased to 80 per cent by application of croton resin concurrently with the benzpyrene.[1]

This augmentation of carcinogenesis, or "cocarcinogenic action," could not be explained as a summated effect of two weak carcinogens, since croton resin gave no decisive evidence of carcinogenicity when applied alone to the skin. Nor could the effect be attributed to a nonspecific irritative action on the part of croton resin, since other skin irritants, such as xylene or turpentine, failed to augment carcinogenesis under similar conditions. It was suggested, therefore, that the cocarcinogenic effect of croton resin constituted a specific reaction distinct from the carcinogenic process itself. Yet the mechanism of cocarcinogenic action remained unexplained.

The present communication is concerned with an extension of this work, certain problems having been chosen for special study as likely to throw light on the nature of cocarcinogenic action and on its relation to the normal process of carcinogenesis. The problems chosen for investigation were the following: 1. The effect of croton resin on carcinogenesis of the skin by concentrated solutions of weak carcinogens; 2. the effect of croton resin on carcinogenesis associated with injections of benzpyrene; 3. the effect of croton resin applied to the skin before or after a limited period of benzpyrene application; and 4. the effect of croton resin on the malignant transformation of skin tumors induced by benzpyrene.

The methods employed in these experiments were essentially the same as those previously used by the author, and need not, therefore, be described again in detail. The white mice and white rats both belonged to fairly homogeneous (though not genetically pure) strains, bred in this laboratory over a period of many years, without any admixture from other sources. All reagents for application to the skin were dissolved in acetone, application being made once a week to a small area of skin in the region of the shoulder blades, the hair being clipped short with scissors prior to each application. Other details of actual experiments can best be left for consideration under the separate headings.

The Effect of Croton Resin on Carcinogenesis of the Skin by Concentrated Solutions of Weak Carcinogens

In previous experiments (Series 1 to 5, Table 1), it was found that the effect on the mouse's skin of the addition of croton oil or resin to benzpyrene treatment differed, depending on whether the benzpyrene was applied as a dilute solution (0.05 per cent) or in a concentrated form (1 per cent). With a dilute solution of benzpyrene, croton resin produced a striking augmentation of carcinogenesis, whereas with a concentrated solution, the effect was insignificant. This difference could be explained in one of two ways: Either the carcinogenic potency of 1 per cent benzpyrene in acetone is already maximal for the mouse's skin, so that further increase in potency was theoretically impossible, or else the mechanism of cocarcinogenic action of croton resin is such that the augmentation represents nothing more than an increase from the artificially lowered potency (produced by dilution) *up to the potential limit for the particular carcinogen.*

The problem was put to the test by determining the influence of croton resin on the carcinogenic action of weak carcinogens applied in concentrated solutions. If the first explanation is correct, croton resin should be able to augment the carcinogenic potency of a concentrated solution of a weak carcinogen as readily as

[1] See Cabot, S., N. Shear, and M. J. Shear. Studies in Carcinogenesis. XI. Development of Skin Tumors in Mice Painted with 3:4-Benzpyrene and Creosote Oil Fractions. Am. J. Path., **16**:301-312. 1940.—Footnote added by Editor.

of a dilute solution of a potent carcinogen; if the second is correct, croton resin should be ineffective when acting with a concentrated solution of any carcinogen, irrespective of whether its potency is high or low.

Four separate groups of mice received weekly applications to the skin of a saturated solution of 1,2,5,6-dibenzanthracene in acetone, of a similar solution together with 0.5 per cent croton oil, of a saturated solution of 1,2-benzanthracene, and of a similar solution together with 0.025 per cent croton resin.[2]

The results of this experiment are summarized in Table I, Series 6 to 9. No significant augmentation of carcinogenesis occurred either with 1,2,5,6-dibenzanthracene or 1,2-benzanthracene, so that, of the two possible explanations of cocarcinogenic action mentioned above, the second appears to be the correct one. Thus, in the former experiment, the two agents were allowed to act together on connective tissue instead of on skin; in the latter, the cocarcinogenic action of croton resin was tested on the skin once again, but with the benzpyrene brought to it in small concentrations through the blood stream, instead of as a dilute solution from the surface.

The effect of croton resin and of turpentine injected subcutaneously in rats together with varying concentrations of benzpyrene.—Twelve groups of rats, 6 in each group, were used for this experiment. The reagents for injection were dissolved in sesame oil, 0.5 cc. of the appropriate solution being injected subcutaneously into the left flank at the commencement of the experiment, after which no further treatment was given. The animals were kept under observation for 9 months, the time of appearance of a tumor being recorded and its size measured periodically. At the end of the experiment, the animals were killed, and any tumors present were examined histologically. The amounts of benzpyrene injected were 2.5 mgm., 0.05 mgm., and 0.001 mgm., respectively. These amounts were injected alone in some groups or together with 50 mgm. of turpentine or 0.005 mgm. of croton resin in others. Three control groups received sesame oil alone, turpentine in sesame oil without benzpyrene, and croton resin in oil without benzpyrene.

With the highest concentration of benzpyrene (2.5 mgm.), the tumor yield was 5/6 with the hydrocarbon alone, 6/6 with benzpyrene plus turpentine, and 6/6 with benzpyrene plus croton resin. The tumors were all spindle cell sarcomas, and their times of appearance and rates of growth did not differ significantly in the 3 groups. Not a single tumor appeared at the site of injection in any of the animals of the other series (*i.e.*, those which received 0.05 mgm. and 0.001 mgm.

TABLE I: RESULTS OF APPLICATION OF CROTON OIL OR RESIN CONCURRENTLY WITH DIFFERENT CARCINOGENS

Series	Reagent for application, dissolved in acetone	Number of mice used	Time of appearance of first tumor, in weeks	Time taken for 50 per cent of survivors to develop tumors, in weeks	Mice with tumors/ survivors, 20th week	Percentage
1.	1% 3,4-benzpyrene	36	12	19½	22/36	61
2.	1% 3,4-benzpyrene and 0.5% croton oil	36	9	17	26/33	79
3.	0.05% 3,4-benzpyrene	36	21½	..	0/34	0
4.	0.05% 3,4-benzpyrene and 0.5% croton oil	36	11½	24	12/32	37
5.	0.05% 3,4-benzpyrene and 0.025% croton resin	20	9½	18	16/20	80
6.	Saturated solution 1,2,5,6-dibenzanthracene	36	12	28	5/33	15
7.	Saturated solution 1,2,5,6-dibenzanthracene and 0.5% croton oil	36	13½	26½	6/32	19
8.	Saturated solution 1,2-benzanthracene	20	..	..	0/16	0
9.	Saturated solution 1,2-benzanthracene and 0.025% croton resin	30	(5½)*	..	0/30	0

* A localized thickening of the skin in one mouse, recorded as a papilloma, proved to be a hyperkeratosis without any outgrowth of epithelium when examined histologically.

THE EFFECT OF CROTON RESIN ON CARCINOGENESIS ASSOCIATED WITH INJECTIONS OF 3,4-BENZPYRENE

The object of this method of approach was to determine whether croton resin was cocarcinogenic under conditions other than those in which both the resin and the benzpyrene are applied to the skin.

Two widely different experiments were undertaken for this purpose, one of which, carried out on rats, was concerned with the effect of croton resin (and also of turpentine) injected subcutaneously, together with different concentrations of benzpyrene; the other, carried out on mice, dealt with the possibility of producing skin tumors at the site of application of croton resin when benzpyrene was injected at a distance.

[2] Croton resin is the active constituent of croton oil, and a solution of 0.025 per cent croton resin corresponds approximately in cocarcinogenic action to a solution of 0.5 per cent croton oil.

of benzpyrene with or without turpentine or croton resin, and the 3 controls). Thus the possibility of cocarcinogenic action on connective tissue is not supported by the present experiment.[3]

The effect of croton resin and of turpentine on the skin of mice periodically receiving intraperitoneal injections of benzpyrene.—Two groups of mice, 20 in each group, received 5 intraperitoneal injections of 0.1 cc. of a 0.1 per cent solution of benzpyrene in sesame oil at monthly intervals, one group receiving, in addition, weekly applications to the skin of 0.025 per cent croton resin in acetone, the other receiving similar applications of 30 per cent turpentine in acetone. The experiment was continued for 25 weeks.

Though two animals in each group developed intraperitoneal tumors (*i.e.,* at the site of injection of the benzpyrene), none showed tumors of the skin at the site of application of the croton resin or turpentine. This experiment was, therefore, also negative.

The Effect of Croton Resin Applied to the Skin Before or After a Limited Period of Benzpyrene Application

It has been shown (6) that when two different chemical carcinogens are allowed to act on the same area of skin for separate periods, the carcinogenic effects are summated, the skin being apparently incapable of distinguishing between one and the other (except quantitatively, of course, when the potencies are different), and responding, therefore, as if one carcinogen were acting all the time. It was thought interesting to investigate whether the same was also true when a carcinogen and a cocarcinogen were allowed to act under similar conditions. Such experiments would show (a) whether a cocarcinogen is capable of producing those biological changes in the skin which characterize the "latent period" of carcinogenesis preceding the actual development of a tumor, even if it cannot produce a tumor by itself, and (b) whether it is capable of "precipitating" the development of a tumor when such biological changes are already present as the result of previous action of a carcinogen.

Two experiments were, therefore, undertaken. In one, the croton resin applications preceded the benzpyrene treatment; in the other, they followed a limited period of benzpyrene treatment.

The effect of preliminary treatment of the mouse skin with croton resin on the subsequent response of the same area of skin to the carcinogenic action of benzpyrene.—The animals used for this experiment were 20 mice which had been receiving weekly applications of croton resin (0.025 per cent in acetone) to the skin for 26 weeks. The original purpose of this experiment was to determine whether croton resin was itself carcinogenic. When it was found that no tumors had appeared after 26 weeks and all the animals were still alive, the croton resin treatment was discontinued and application of 1 per cent benzpyrene in acetone was substituted.

The results of this experiment can briefly be summarized as follows: No tumors arose during the first 9 weeks from the commencement of benzpyrene treatment; from then onwards, warts began to appear at the site of application, and, after 16 weeks (42 weeks from the beginning of the original experiment), 10 out of 19 survivors bore tumors.

When compared with numerous control experiments from other series (*i.e.,* in which 1 per cent benzpyrene in acetone was applied at weekly intervals to similar mice, without preliminary treatment of any sort), the above results fail to indicate any significant shortening of the latent period as the result of the preliminary applications of croton resin. The time taken for 50 per cent of the animals to develop tumors was admittedly a little shorter than in control groups (16 weeks as compared to 18 to 19½ weeks); on the other hand, the time taken for the first tumor to appear (9 weeks) was well within normal limits (5 to 13 weeks).

It may be concluded, therefore, that little or no effect is produced by prolonged application of croton resin, which might suggest the development of those biological changes which are characteristic of the latent period of carcinogenesis.

The effect of croton resin on mouse skin previously treated with benzpyrene for a limited period.—One hundred mice received 8 weekly applications to the skin of a 1 per cent solution of benzpyrene in acetone, by which time one animal had already developed a tumor at the site of application. This animal was discarded. In the remaining mice, the benzpyrene treatment was discontinued, and the animals were divided into 3 groups. In one, the previously treated skin was painted with 0.025 per cent croton resin in acetone at weekly intervals; in the second, the same area of skin was painted with 30 per cent turpentine in acetone; while in the third, acetone alone was applied as control. The painting was continued for 22 weeks (*i.e.,* 30 weeks from the commencement of

[3] In analogous experiments Sall and Shear found that when appropriate dosages and conditions were employed the basic fraction of creosote oil promoted the development of tumors following the subcutaneous injection of 0.1 mgm. of benzpyrene in mice. These positive results support the conception of cocarcinogenic action. See: Sall, R. D., and M. J. Shear. Studies in Carcinogenesis. XII. Effect of the Basic Fraction of Creosote Oil on the Production of Tumors in Mice by Chemical Carcinogens. J. Nat. Cancer Inst., 1:45-55. 1940.

the experiment), after which the animals were left untreated for a further 6 weeks and then killed for histological examination of the tumors.

The results of this experiment (Table II) may be summarized as follows: In the acetone control series, 5 of the animals (representing 18 per cent of survivors) developed tumors at the site of application; in the turpentine series, the number of animals with tumors was 11 (representing 44 per cent); while in the croton resin series, as many as 25 animals bore tumors at the site of application (representing 86 per cent of survivors). In the control and croton resin series, the tumors were small pedunculated papillomas which grew slowly and only a small proportion of these became malignant, whereas in the turpentine series most of the tumors were sessile from the start, grew rapidly, and tended to become malignant early.

Table II: The Effects of Croton Resin, Turpentine, and Acetone on Mouse Skin Previously Treated with 3,4-Benzpyrene for 8 Weeks

	Croton resin	Turpentine	Acetone
A. Number of mice used	30	30	30
B. Number of survivors at end of the experiment	29	25	28
C. Number of mice which developed tumors	25	11	5
D. Percentage (C:B)	86%	44%	18%
E. Times of appearance of tumors (in weeks)	2, 3, 5, 6, 8, 8, 9, 9, 10, 10, 11, 11, 11, 11, 13, 14, 14, 15, 15, 15, 16, 16, 17, 18, 22.	1, 5, 6, 8, 12, 15, 16, 17, 19, 22, 25.	1, 3, 3, 20, 27.
F. Analysis of tumors: Number of animals in which the tumors have			
1. Regressed	6	0	2
2. Remained small	8	2	1
3. Grown progressively without becoming malignant	8	4	0
4. Become malignant	3	5	2

The two last experiments show, therefore, that whereas application of croton resin *prior* to benzpyrene treatment has little or no demonstrable effect, application of croton resin *following* a limited period of benzpyrene treatment leads to a pronounced increase in the development of tumors. The results with turpentine are much less striking, while the evidence about malignancy appears rather anomalous.

The Effect of Croton Resin on the Malignant Transformation of Skin Tumors Induced by Benzpyrene

In view of the above results, it was considered important to investigate more carefully whether croton resin or turpentine had any effect on the transformation of a benign into a malignant skin tumor.

The experimental demonstration of such an effect presents certain practical difficulties, owing to the great individual variability in the rate of appearance of benign tumors of the skin in mice following applications of a chemical carcinogen, and the even greater variability in the time taken for such tumors to become malignant. The following experiment was designed to overcome, to some extent, these inherent practical difficulties.

Weekly applications of a 1 per cent solution of benzpyrene in acetone were made to the skin of 100 mice. As soon as any animal developed a wart, it was segregated and given an identification mark. The first animal with a tumor was placed in group A, the second in group B, the third in group C, the fourth in group A again, and so on, in rotation, until all the animals were separated in one or other of the 3 groups. By this means, each group contained the same proportion of animals with early, intermediate, and late developments of warts. Once an animal was segregated, the benzpyrene treatment was stopped, and acetone (in the case of group A), croton resin (in the case of group B), and turpentine (in the case of group C) were substituted for application to the same area of skin. Records were kept of the dimensions of the tumors at regular intervals, and 10 weeks after the appearance of a tumor, the animal in question was killed for histological examination of that tumor. In this way, it was possible to determine, in spite of the individual variability in response to carcinogenic action of the preliminary benzpyrene treatment, the average tendency towards malignant transformation in the 3 groups.

The results of this experiment were as follows (Table III): In the acetone control series, malignant tumors were present in 7 out of 17 survivors (41 per cent), in the turpentine series, in 6 out of 15 survivors (40 per cent), and in the croton resin series, in 11 out of 18 survivors (61 per cent). When the rapidly growing tumors, already bordering on malignancy, are also taken into account, the results appear more decisive. The numbers of mice in which the tumors regressed or remained as small warts or grew slowly, compared to those in which the tumors grew rapidly and either bordered on or actually reached malignancy, were 9 and 8 in the acetone control series, 6 and 9 in the turpentine series, and 4 and 14 in the croton resin series; *i.e.*, a ratio of 1:0.9, 1:1.5, and 1:3.5, respectively.

TABLE III: EFFECTS OF CROTON RESIN, TURPENTINE, AND ACETONE ON WARTS PREVIOUSLY INDUCED BY APPLICATIONS OF 3,4-BENZPYRENE

	Croton resin	Turpentine	Acetone
1. Number of mice used	20	20	20
2. Number of survivors at end of the experiment	18	15	17
3. Number of mice in which tumors regressed	1	6	8
4. Number of mice in which tumors grew but remained benign	3 (4)	0 (6)	1 (9)
5. Number of mice in which tumors reached early or probable malignancy *	3	3	1
6. Number of mice in which tumors became definitely malignant †	11 (14)	6 (9)	7 (8)
7. Ratio of 3 + 4 to 5 + 6	1:3.5	1:1.5	1:0.9

* Histological evidence of downward growth, without invasion of muscle.
† Histological evidence of invasion of muscle.

It is unfortunate that, in view of the complexity of the experiment, larger numbers of animals could not have been used. The results, nevertheless, suggest (though they cannot be considered as conclusive) that croton resin facilitates the conversion of warts from the benign to the malignant state. The results concerning the action of turpentine are inconclusive.

DISCUSSION

In view of recent developments in our knowledge of the mechanism of carcinogenesis, and the confusion which must inevitably arise from endless repetition of such phrases as "the production of those biological changes which represent the latent period of carcinogenesis" or "the precipitation of a tumor at a site previously rendered preneoplastic," it has become necessary to adopt a special terminology. It is hoped that the use of the following terms will facilitate clarity of expression in discussion on the subject:

Anticarcinogenic action: The inhibition of the process of carcinogenesis. The term was introduced by the author (2, 3, 4) to describe the action of dichlorethylsulfide, cantharidin, etc.

Cocarcinogenic action: The augmentation of carcinogenesis by a noncarcinogenic agent.[4] This occurs when the appropriate agent is applied concurrently with a carcinogen which is acting under suboptimal conditions. The term was first introduced by Shear (12) for a certain noncarcinogenic fraction of tar and has been adopted by the present author for the action of croton resin (5).

Precarcinogenic action: The production of a preneoplastic condition. Such an effect would be demonstrable by a shortening of the latent period of carcinogenesis in subsequent treatment with a carcinogen, or by preparing the ground for the subsequent action of an epicarcinogenic agent (see below).

Epicarcinogenic action: The production of tumors in a tissue previously rendered preneoplastic.

Metacarcinogenic action: The conversion of a benign into a malignant tumor.

When a chemical carcinogen is applied repeatedly to the skin of a susceptible animal, a series of biological changes develops in an orderly sequence (the time relationship varying, however, from animal to animal). At least three well-recognized stages are involved: (a) the preneoplastic stage (or latent period of carcinogenesis), (b) the wart or papilloma stage, and (c) the stage of malignancy. In the first stage, no tumors are yet detectable, but the histological appearances of the skin (epithelial hyperplasia, inflammatory changes in the corium, etc.), though resembling in many respects those obtained with noncarcinogenic irritants, seem to possess certain specific features (8, 10). The essential characteristic of the second stage is that the warts which develop are usually multiple and arise as minute foci, suggesting that their origin is, in each case, from a single cell. The third stage (malignancy) develops, in the majority of cases, from one or other of the existing warts, the remainder of the painted area of skin being rapidly included in the malignant mass by invasion, and probably not by malignant transformation of the surrounding tissue.

Precarcinogenic action may, therefore, be considered as the conversion of normal skin into that of stage (a); epicarcinogenic action as the conversion from stage (a) into stage (b), and metacarcinogenic action as the conversion from stage (b) into stage (c), while anticarcinogenic action represents interference with epicarcinogenic action only, since it has been shown (3) that, when applied in subulcerative concentrations, neither the preneoplastic state nor the subsequent

[4] The phrase "by a noncarcinogenic agent" has been added by the Editors.

growth of warts, once these have been established, is inhibited by the anticarcinogens so far studied.

Since any one carcinogenic agent is capable of producing all three effects (pre-, epi-, and metacarcinogenic actions), it is generally assumed that these represent three inseparable stages of one single carcinogenic process. This unitarian conception of carcinogenesis is not supported, however, by the results described in the present communication.

Croton resin is not carcinogenic by itself, nor is it capable of producing, to any demonstrable extent, those preneoplastic changes which characterize the latent period of carcinogenesis. Yet, when applied to skin which has already been rendered preneoplastic by other means, it is capable of precipitating the development of warts in a high proportion of cases, and furthermore, when allowed to act on warts already established, it seems able to facilitate their conversion into malignant tumors.

These results can be taken as evidence of a dissociation of the process of carcinogenesis into several component parts, the whole process constituting, as it were, a chain reaction of essentially independent processes. That this interpretation is valid when applied to the normal process of carcinogenesis is evident from a consideration of recent results by Rous and his associates (7, 11).

These authors have drawn attention to the differences in the response of the skin of the mouse and rabbit respectively to the carcinogenic action of tar. While papillomas develop fairly readily in both species, those in the mouse tend to grow progressively, even after discontinuing the tarring, whereas those arising in the rabbit usually regress under similar conditions. Moreover, whereas the mouse papilloma has a strong bias towards malignancy, that of a rabbit becomes malignant only with difficulty, even under optimal conditions. These authors demonstrated, however, that in spite of the strong tendency to regression, tar papillomas in the rabbit possess all the valid criteria of neoplasia, since, after complete regression, they could be made to reappear by a variety of influences (renewed tarring, wound healing, and turpentine).

Thus, even in the normal process of carcinogenesis, the separate component parts (pre-, epi-, and metacarcinogenic actions) develop to different relative degrees in the two species. In the mouse, all 3 processes progress more or less rapidly, the impetus towards final malignancy being fairly pronounced even at an early stage, whereas in the rabbit, the precarcinogenic effect is extremely pronounced (the latent period being sometimes as short as 2 weeks). The epicarcinogenic effect is also pronounced, though continued encouragement is required for the warts to continue to grow, while the metacarcinogenic effect is relatively feeble.

Evidence in favor of a dissociation of the process of carcinogenesis is, therefore, of two kinds: 1. The existence of certain substances which are capable of producing part, but not all, of the changes leading to neoplasia, and 2. the fact that the individual stages of carcinogenesis develop with relatively different degrees of impetus in different species. As a basis for further research, it may be helpful to present this new conception of carcinogenesis in a more concrete form:—

1. Precarcinogenic, epicarcinogenic, and metacarcinogenic actions represent independent processes in carcinogenesis.

2. True carcinogens are capable of producing all three actions, though to different degrees in different species.

3. Croton resin differs from true carcinogens in lacking the power of precarcinogenic action, but resembles them in possessing both epi- and metacarcinogenic action.

4. It is possible that other kinds of incomplete carcinogens may exist, possessing, for instance, metacarcinogenic but not pre- or epicarcinogenic action.

5. The varying response to carcinogenesis of different tissues may possibly be attributable to a deficiency in one part of the chain reaction, rather than to a greater or lesser responsiveness to carcinogenesis as a whole.

But what then does cocarcinogenic action represent in this scheme of the mechanism of carcinogenesis?

When the cocarcinogenic action of croton resin was first described (5), some consideration was given to the possibility that the effect was part of a normal carcinogenic process; *i.e.,* that the action was due to a summated effect on the part of two weak carcinogens, dilute benzpyrene and croton resin. This view was rejected because no evidence of carcinogenic action on the part of croton resin was obtainable when this substance was applied alone to the skin, while the possibility that croton resin was too weak a carcinogen to produce tumors by itself, yet strong enough to act in conjunction with another weak carcinogen in the above manner, was shown to be untenable on theoretical grounds. Final refutation of this view concerning the mechanism of cocarcinogenic action is now available. If it were correct, one would have expected croton resin to augment the carcinogenic action of a saturated solution of a weak carcinogen as readily as of a dilute solution of a potent carcinogen. Yet this was found not to be the case.

This negative conclusion does not lead one very far, however, and the other results, described in the present communication, present equally negative evidence, and cannot do more, therefore, than merely indicate vaguely the probable nature of cocarcinogenic action.

The negative results obtained when benzpyrene was injected at a distance from the site of application of the croton resin are probably not very significant, since the concentration of benzpyrene actually reaching the painted area of skin may well have been too low for the cocarcinogenic effect of the croton resin to manifest itself. It is interesting to note in this connection that, according to Beck (1), intraperitoneal injections of small amounts of benzpyrene in mice failed also to augment the neoplastic response of the skin to cauterization, x-rays, and ultraviolet irradiation, though, from earlier experience, tar appeared to be effective. In recent investigations which have not yet been published, by Dr. Schontal and the author, it was found possible to detect and measure the small amounts of benzpyrene present in the circulating blood of the mouse following subcutaneous and intraperitoneal injection of the hydrocarbon. When more data from these investigations are available, it may become possible to establish the conditions of injection required for an adequate amount of the benzpyrene to reach the skin through the blood stream.

The failure to demonstrate any cocarcinogenic action on the part of croton resin when injected subcutaneously into rats in conjunction with benzpyrene, though more convincing than the above experiment, must also be interpreted with caution.[5] It is known (9) that tumors induced subcutaneously develop at some little distance from the actual site of injection of the carcinogen, and since in the present experiment the croton resin and the benzpyrene were injected together as one solution, it is possible that the resin may not have been able to diffuse out as far as the benzpyrene and may, therefore, have failed to reach the actual zone of potential carcinogenesis. The conditions of the experiment differed also from those in which cocarcinogenic action was demonstrated on the skin since, in the latter, a low concentration of benzpyrene was applied repeatedly at short intervals, so that the tissue was always under the influence of small amounts of the carcinogen, whereas in the former only one injection was given, and, in the case where a small amount of benzpyrene was injected, probably none was present at the time when the cocarcinogenic action of the croton resin could theoretically have become manifest.

The most significant experiment was that in which the croton resin was applied to the skin in conjunction with 1,2,5,6-dibenzanthracene and 1,2-benzanthracene respectively, and, apart from the conclusions which have already been reached from this experiment, the results also help to throw a little light on the mechanism of cocarcinogenesis.

The simplest conception of cocarcinogenic action would be that it was merely a variant of epicarcinogenic action, on the supposition that the dilute benzpyrene produced the preneoplastic state, while the epicarcinogenic effect was carried out jointly by the dilute benzpyrene and the croton resin. If this were the case, one would have to assume, first, that the precarcinogenic action of benzpyrene is less influenced by dilution than its epicarcinogenic action and, second, that the low carcinogenic potency of dibenzanthracene and the still lower potency of benzanthracene are due primarily to deficiencies in precarcinogenic action. Until these assumptions are confirmed experimentally it is not possible to say whether this simple explanation of cocarcinogenic action is correct or not.

An entirely different explanation of the mode of action of cocarcinogenesis would be to suppose that croton resin merely facilitated the entry of the carcinogen into the cell so that a small number of molecules of the hydrocarbon, applied to the surface, would still have a reasonable chance of acting on the cell. This would account for the failure of croton resin to augment carcinogenesis in the case of concentrated solutions of carcinogens, irrespective of whether their potencies are high, medium, or low. This interpretation implies, however, that cocarcinogenic action is an entirely different process from pre-, epi-, and metacarcinogenic action. No decision can be made at the present stage as to the likelihood of this explanation being the right one.

In conclusion, it is necessary to stress the practical implications of the present results in relation to clinical and preventive medicine. The elucidation of the specific chemical nature of carcinogenic hydrocarbons and the demonstration that most of the ordinary skin irritants are not in themselves carcinogenic, have tended to distract clinical attention from the possibility that irritation might play a part in the development of tumors in man. However, the fact that certain noncarcinogenic irritants are capable of producing cocarcinogenic, epicarcinogenic, and metacarcinogenic effects introduces new conceptions of possible extraneous factors of a noncarcinogenic nature influencing tumor development in man.

Summary

The effect of croton resin on carcinogenesis was studied under varying conditions, in order to determine the nature of cocarcinogenic action (the augmentation of carcinogenesis which occurs when croton resin is applied to the skin concurrently with a dilute solution of 3,4-benzpyrene) and its relation to the normal process of carcinogenesis.

No cocarcinogenic effect was observed when the croton resin was applied to the skin and the benzpyrene was injected at a distance (intraperitoneally);

[5] See footnote 3.

nor was it possible to augment the carcinogenic effect of benzpyrene on subcutaneous tissues, by injection of croton resin together with the benzpyrene.

While augmentation of carcinogenesis was very pronounced when croton resin was applied to the skin concurrently with a *dilute* solution of a potent carcinogen (3,4-benzpyrene), none was observed with *concentrated* solutions of different carcinogens, irrespective of whether their potency were high (3,4-benzpyrene), moderate (1,2,5,6-dibenzanthracene), or very low (1,2-benzanthracene).

Preliminary treatment with croton resin for a period of 26 weeks failed to influence significantly the response of the mouse's skin to subsequent applications of benzpyrene. On the other hand, croton resin applied to the skin subsequent to a limited period of benzpyrene treatment led to a striking increase in the development of tumors.

Croton resin applied to papillomas already established appeared to facilitate their conversion to malignancy.

From consideration of these results, the suggestion is put forward that the three phases of carcinogenesis—(a) the development of the preneoplastic phase (latent period), or *precarcinogenic action,* (b) the conversion of this into the wart stage, or *epicarcinogenic action,* and (c) the malignant transformation of these warts, or *metacarcinogenic action*—are probably not simply stages of one single carcinogenic process, but independent processes. The carcinogenic hydrocarbons possess all three actions; croton resin possesses only the second and third, and cannot, therefore, produce tumors by itself.

No precise knowledge is yet available as to the nature of cocarcinogenic action, but two possible modes of action are discussed.

Attention is also drawn to the clinical implications of the existence of cocarcinogenic, epicarcinogenic, and metacarcinogenic actions on the part of noncarcinogenic agents in man.

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Failure to Induce Sarcoma in Rats with Wheat Germ Oil Preparations

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The production of neoplasms in albino rats by the feeding of crude wheat germ oil, made by ether extraction, has been reported by Rowntree and colleagues (6, 11, 12, 13). Oils made by other methods than ether extraction did not give rise to tumors. The effect was not strain-specific, since abdominal sarcomas were produced in rats of the Wistar, Buffalo, and Yale strains. These rats were maintained on a special diet throughout the experiment.

Various attempts have been made to repeat this work. Using rats of the Wistar and Sprague Dawley strains, Carruthers (2) obtained negative results. Halter (9) using Wistar rats likewise obtained negative results after feeding 1 cc. of oil daily for 12 months. Dingemanse and van Eck (4) attempted to concentrate the agent. They first extracted the wheat embryo with petroleum ether removing about 95 per cent of the lipid fraction, and then with ether. The oils obtained by this method and by ether extraction alone did not produce tumors in Wistar-Piebald crossbred rats. Rowntree and associates used peroxide-free ether for their extractions. Day, Becker, and McCollum (3), however, investigated the possibility that tumors might be due to the effect of peroxide present in ether, with negative results. The relative amount of vitamin E was reported by the original workers not to be an important factor. Further studies along this line were made by Evans and Emerson (7) using rats (not albino) of the Long-Evans strain. They obtained no tumors. Other negative results have been published by Auchincloss and Haagensen (1), Ginzton and Connor (8), Rider (10), Dittmar and Burschkies (5), and Sannié and Truhaut (14).

For over 2 years we have been carrying out feeding experiments with the Wistar and Slonaker strains of albino rats on several different wheat germ oil preparations. We have fed a medicinal wheat germ oil (designated MO), and an ether-extracted wheat germ oil (EEO), made exactly according to the procedure (13) described by the original workers. We made an ether extraction of wheat germ cake (WGCO) from which medicinal oil had been expressed, hoping to obtain by this method a concentrated solution of the agent. We also dissolved medicinal oil in ether and treated it as if it had been ether-extracted (ETMO).

Materials

The oils were prepared at The Abbott Laboratories and at Northwestern University, and the biological work was conducted at the Collis P. Huntington Memorial Hospital.[1]

Ether-extracted wheat germ oil, EEO.—The preparation of this oil from fresh wheat germ was an accurate duplication of the original (13). Some of the EEO was made by decantation and the rest by percolation as recommended.

Oil made by the ether extraction of wheat germ cake, WGCO.—Fresh wheat germ cake from which medicinal oil had been expressed was percolated with ethyl ether. The ether extract was filtered and further treated as in the preparation of the EEO. From 1,000 pounds (453.6 kg.) of wheat germ cake 31 pounds of solvent-free oil were obtained.

Ether-treated medicinal wheat germ oil, ETMO.—Abbott medicinal wheat germ oil was dissolved in ether and the same procedure followed as in the preparation of EEO. In a typical experiment 1,307.6 gm. of oil was dissolved in 1.5 liters of ethyl ether and allowed to stand several days at room temperature. The solution was then washed twice with about half volumes of 4 per cent NaOH solution to remove the free fatty acids. Sometimes emulsions were obtained which alcohol failed to break readily; in such cases NaCl was also added. Otherwise no changes were made in the original directions (13). After the ether was removed 1,132 gm. of oil were obtained. The yield was sometimes lower when troublesome emulsions were encountered.

Biological Assay

Albino rats of the Slonaker strain were used in the first experiments. They were fed on a stock diet, the formula for which was identical with that described by Rowntree and associates (13), to which greens were added once a week. The oils were mixed intimately with the diet. During the first 2 weeks, 300 cc. of oil

[1] The authors wish to thank the Abbott Laboratories, North Chicago, Illinois, which have generously supported this research. We wish especially to express our appreciation to Dr. E. H. Volwiler, Vice-President and Director of Research, and Mr. Carl Nielsen, Director of Nutritional Research. They furnished the medicinal wheat germ oil which was used for the controls in the feeding experiments and for the ether treatment. The EEO and WGCO were prepared for us by the Abbott Laboratories.

were added to 675 gm. of the diet; subsequently 300 cc. of oil were added to each kg. of the diet. Sixteen rats were started in December, 1937; 8 males on MO; 6 males and 2 females on EEO. Each of these groups comprised 4 rats just weaned and 4 rats weighing between 100 and 200 gm. These groups received oil for 6 months; oil was then not available for 2 months, following which it was given for 7 months. In June, 1938, two groups each of 8 male rats were started on the other two oils (WGCO and ETMO) and oil was given for 10 months. All rats were weighed and examined by abdominal palpation at regular intervals, and for the first few weeks the food was weighed and the oil intake calculated. The calculated consumption of the various oils varied between 2.5 and 4.0 cc. daily per rat. A summary of the results is given in Table I.

The second part of the experiment was done using 32 Wistar rats between 8 and 10 weeks of age. Groups of 8 (4 males and 4 females) were given MO, EEO, EGCO, and no oil, with the stock diet exactly as in the earlier trials. The oils were administered continuously for 6 months. All rats gained weight normally and no abdominal masses or other abnormalities were noted. All but two of the rats have lived 11 months since the beginning of the experiment; two died without malignancy and the remainder show no evidence of tumors.

TABLE 1: RESULTS OF FEEDING PREPARATIONS OF WHEAT GERM OIL

Oil given	Number of rats	Mean daily oil consumption per rat (cc.)	Median duration of life, months	Number surviving, months 6	11	18	Number showing evidence of malignancy
SLONAKER RATS							
MO *	8	2.5	8.5	6		1	0
EEO †	8	3.0	8.5	6		2	0
WGCO ‡	8	3.6	14.0	7		3	0
ETMO §	8	4.0	16.0	8		3	0
WISTAR RATS							
MO *	8	4.2	>11		7		0
EEO †	8	4.7	>11		8		0
WGCO ‡	8	4.3	>11		7		0
No oil	8	0.0	>11		8		0

* MO = medicinal wheat germ oil.
† EEO = ether-extracted wheat germ oil.
‡ WGCO = ether extraction of wheat germ cake.
§ ETMO = ether-treated medicinal wheat germ oil.

Careful post-mortem examination failed to reveal malignant tumors in the abdomen or elsewhere in any of the animals. The commonest finding was chronic cecitis, which occurred frequently in the rats of our colony during the period of these experiments. This was found in greater or less degree in 9 of the 22 rats, comprising 3 in the group given medicinal oil and 2 in each of the other groups. Microscopic examination of tissue was made in all of these cases, and showed only chronic inflammatory changes in the cecal wall, with edema and inspissated fecal material. Several other animals were found to have freely movable abdominal masses during life, similar to those in animals with proven cecitis, which disappeared after one to four months. Five rats were found at post-mortem to have blood in the terminal ileum and below, without obvious bleeding points; 3 of these had received medicinal oil. Three rats died with middle ear disease and labyrinthitis.

DISCUSSION

The Slonaker rats receiving medicinal oil and ether-extracted oil did not, on the whole, survive as long as those fed the other two oils. These other oils were made assuming that the agent might be (a) not removed from wheat germ by customary procedures used in the preparation of medicinal oil, but only by ether, or (b) produced by the action of ether upon components present in medicinal oil. The differences in rate of survival were probably fortuitous, as they represent rats chosen from the colony at different dates, and do not appear in the experiments with Wistar rats.

SUMMARY

Thirty-two Wistar and 32 Slonaker albino rats were fed 2.5 to 4.7 cc. daily of four wheat germ oils, including medicinal oil and ether-extracted oil. These preparations were given for several months. No malignant tumors were found.

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The Effect of Carcinogenic Hydrocarbons and Related Compounds on the Autoxidation of Oils*

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The theory that the carcinogenic hydrocarbons might exert an influence on the tissues by affecting the lipid part of the cell is suggested by the fact that these compounds are insoluble in water but are soluble in most fats and fat solvents. However, very little is known regarding the cellular metabolism of fats, and it is difficult to find a satisfactory system on which to test this theory. One possible mode of action of the carcinogens on fat metabolism might be through their effect on phospholipid oxidation. Rusch and Kline (6) have demonstrated that the oxidation of phospholipids in the presence of glutathione, cysteine, ascorbic acid, thiamin, riboflavin, or pyridoxine can be inhibited by carcinogenic chemicals. It is not known, however, whether the antioxygenic action of the carcinogens is specific for catalyzed phospholipid oxidation or whether other types of fat oxidation are also affected by the hydrocarbons. Accordingly, the following experiments were designed to determine the effect of the carcinogenic chemicals and related compounds on the autoxidation of fats.

Methods

Oils were allowed to oxidize in the presence or absence of various chemicals and the amount of oxidation determined by three methods. Corn oil was used for the first two methods of estimating autoxidation. The various chemicals to be tested were added to the oil at levels of 0.01 and 0.05 per cent and the mixtures exposed to air in Petri dishes at 37° C. for 10 days. At the end of this period the amount of autoxidation was determined. The first method consisted of a modification of the Kreis test as described by Walters, Muers, and Anderson (7). Three cc. of oil diluted with amyl acetate were transferred to a colorimeter tube and 2 cc. of trichloracetic acid solution in amyl acetate (1 gm. per 0.38 cc.) were added and mixed. To this 1 cc. of a 0.5 per cent solution of phloroglucinol in amyl acetate was then added and again thoroughly mixed. A blank was carried through the same steps except that no phloroglucinol was added to the amyl acetate. After standing 30 minutes at room temperature to allow the color to develop, readings were made at 540 mμ with an Evelyn colorimeter. Dilutions of 1 part of corn oil in 100 of amyl acetate were used since this gave a color intensity within the region of maximum accuracy of the colorimeter, and Beer's law was found to hold over the entire range at this dilution. The amount of color depends upon the epihydrin aldehyde formed by oxidation and is proportional to L, or $2 - \log G$, G being the galvanometer reading. As a second method of estimating oxidation, the peroxide numbers were determined by the method of French, Olcott, and Mattill (2).

The Kreis test and peroxide determinations depend upon the formation of products of oxidation and are not a measure of the total oxidation. Therefore, the total oxygen consumption was followed by the manometric method. Two cc. of oil were added to each flask except for ethyl linoleate which was used at 1 cc. levels. With the cod liver oil and lard cod liver oil mixture (1:1), the hydrocarbons and other chemicals were dissolved directly in the oil and 0.005 per cent of copper oleate (8) was added to shorten the induction period of oxidation. No catalyst was used with the corn oil or ethyl linoleate preparations and the carcinogens were dissolved in ether before mixing with these oils. Suction was then applied and the ether removed at 90° C. with continuous vigorous shaking. The presence of naturally occurring antioxidants in corn oil was responsible for a slow rate of oxidation and since the results with this oil were so variable, it was necessary to remove these inhibitors. This was accomplished by placing a flask with corn oil in an oven at 100° C. and bubbling air through for 24 hours. The oil was kept at the same temperature for another 24 hours during which time a stream of nitrogen was passed through it. This treatment resulted in the destruction of most of the inhibitors but was also responsible for other oxidation changes in the oil (5). Gas was liberated for varying periods of time from certain samples of both fresh and heated corn oil. This was prevented in some cases by removing all dissolved gas under a high vacuum. The rate of oxidation was measured for a

* This investigation was aided by a grant from the Jonathan Bowman Fund for Cancer Research.

period of 3 to 10 hours with the aid of a Warburg apparatus at 38° C. Except for 9,10-dimethyl-1,2-benzanthracene, which was prepared in our laboratory, the carcinogens and other compounds added to the oils were crystalline materials obtained commercially. The ethyl linoleate was also prepared in our laboratory. Each experiment was repeated 4 to 8 times.

Results

The effect of various compounds on the autoxidation of the corn oil which had been allowed to stand in a warm room at 37° C. for 10 days is given in Table I. It will be noted that while there is some variation in the values of L, the difference is too small to ascribe antioxidant properties to any of the compounds except hydroquinone. Essentially the same results were obtained when the oxidation was checked

Table I: The Effect of Various Compounds on the Autoxidation of Corn Oil.

Compound	L values Amounts used		Peroxide No. Amounts used	
	0.01 per cent	0.05 per cent	0.01 per cent	0.05 per cent
Control	0.679	0.659	27.0	27.0
3,4-Benzpyrene	0.733	0.721	31.1	25.1
1,2,5,6-Dibenzanthracene	0.683	0.581	29.9	25.6
Phenanthrene	0.606	0.751	29.7	30.6
Cholesterol	0.688	0.688	29.4	25.9
Desoxycholic acid	0.733	0.683	28.3	28.9
Anthracene	0.673	0.569	30.9	25.6
Dihydroxydiphenyl	0.673	0.648	26.4	25.6
Benzoyl peroxide	0.716	0.756	29.6	36.5
Alloxan	0.694	0.733	23.7	17.4
Hydroquinone	0.553	0.347	16.3	6.9

by the peroxide numbers. It will be noted that alloxan gave a slight inhibiting effect whereas benzoyl peroxide accelerated oxidation somewhat. This action of benzoyl peroxide is similar to that of perbenzoic acid which acts as a pro-oxidant, presumably by destroying the naturally occurring antioxidants present in the oils (4). Other changes were not consistent in all experiments.

In Table II is listed the effect of various substances on the oxygen uptake of cod liver oil. Hydroquinone and alloxan inhibited while benzoyl peroxide accelerated oxidation. Increasing the amount of 9,10-dimethyl-1,2-benzanthracene resulted in a stimulation of oxygen consumption of the lard cod liver oil mixture (Table III). Similar results were obtained with methylcholanthrene on a few runs but such effects were not consistent. With the exception of hydroquinone, most of the chemicals accelerated the oxidation of corn oil (Table IV). This was especially true with alloxan. All samples of oil did not show this stimulating effect, however.

Ethyl linoleate was employed in an effort to minimize the variable results obtained with the crude oils. This ester autoxidizes at a rapid rate and the results of four separate runs are shown in Table V. Freshly prepared ethyl linoleate oxidized at a slower rate than samples which were a few days old. Marked inhibition was always obtained with hydroquinone and, with the exception of one experiment, the same was

Table II: The Effect of Various Compounds on the Autoxidation of Cod Liver Oil *

Compound (0.4 mgm. per flask)	Oxygen consumption (cu. mm.)				
	1 hr.	2 hr.	3 hr.	4 hr.	5 hr.
Control	80	157	208	278	348
20-Methylcholanthrene	81	144	...	295	...
1,2,5,6-Dibenzanthracene	86	154	...	277	...
3,4-Benzpyrene	81	145	...	294	...
9,10-Dimethyl-1,2-benzanthracene	88	157	...	293	...
Benzanthracene	78	153	210	...	334
Phenanthrene	86	146	210	...	330
Anthracene	91	150	208	...	327
Benzanthraquinone	84	146	220	...	340
Anthraquinone	74	134	210	...	342
Dihydroxydiphenyl	77	148	200	...	316
Desoxycholic acid	74	139	198	...	336
Benzoyl peroxide	84	146	220	...	374
Alloxan	57	105	147	...	235
p-Dimethylaminoazobenzene	68	136	218	..	340
Ascorbic acid	71	130	204	...	310
Hydroquinone	30	60	79	...	123

* 2 cc. cod liver oil per flask—0.005 per cent copper oleate added.

Table III: The Effect of Various Compounds on the Autoxidation of Lard-Cod Liver Oil Mixture (1:1) *

Compound (2 mgm. per flask)	Oxygen consumption (cu. mm.)		
	1 hr.	2 hr.	3 hr.
Control	130	273	588
20-Methylcholanthrene	132	291	572
1,2,5,6-Dibenzanthracene	126	272	584
3,4-Benzpyrene	127	261	540
9,10-Dimethyl-1,2-benzanthracene	144	308	668
Hydroquinone	25	38	85

* 2 cc. mixture per flask—0.005 per cent copper oleate added.

true for ascorbic acid. In the latter case, however, an initial inhibitory phase of 2 hours was followed by a period of stimulation. The antioxygenic qualities of ascorbic acid on oils have been described by Golumbic and Mattill (3). A mild inhibitory effect was also observed with dibenzanthracene. A marked stimulation was noted with alloxan and a less conspicuous acceleration was observed with aminoazotoluene and desoxycholic acid. In general, the oxidation of freshly prepared ethyl linoleate was accelerated by most hy-

drocarbons while that of older samples was inhibited (Table V).

TABLE IV: THE EFFECT OF VARIOUS COMPOUNDS ON THE AUTOXIDATION OF CORN OIL *

Compound (0.1 mgm. per flask)	Oxygen consumption (cu. mm.) 1 hr.	2 hr.	3 hr.
Control	65	159	312
3,4-Benzpyrene	104	233	382
20-Methylcholanthrene	102	222	364
1,2,5,6-Dibenzanthracene	47	148	300
9,10-Dimethyl-1,2-benzanthracene	74	197	346
Anthracene	88	194	331
Phenanthrene	88	195	330
Cholesterol	95	210	357
Alloxan	126	255	405
Benzoyl peroxide	98	214	361
p-Dimethylaminoazobenzene	78	175	300
Aminoazotoluene	82	182	308
Hydroquinone	6	14	17

* 2 cc. corn oil per flask.

TABLE V: THE EFFECT OF VARIOUS COMPOUNDS ON THE AUTOXIDATION OF ETHYL LINOLEATE *

Compound (0.5 mgm. per flask)	Oxygen consumption 4 hours—cu. mm. Age of oil from time of preparation 1 day	2 days	4 days	20 days
Control	175	410	460	624
3,4-Benzpyrene	208	505	300	324
1,2,5,6-Dibenzanthracene	156	214	312	288
20-Methylcholanthrene	250	505	204	487
9,10-Dimethyl-1,2-benzanthracene	328	515	369	581
Aminoazotoluene	401	661	586	589
Phenanthrene	289	529	384	359
Benzanthracene	306	495	355	572
Desoxycholic acid	462	578	596	494
Benzoyl peroxide	272	497	378	603
Alloxan	2,821	1,920	2,102	...
Hydroquinone	0	12	10	54
Ascorbic acid	20	129	48	631

* 1 cc. ethyl linoleate per flask.

DISCUSSION

These experiments demonstrate that the oxidation of oils and phospholipids differs in at least two respects. Carcinogenic hydrocarbons and related compounds consistently cause a marked inhibition of catalyzed phospholipid oxidation whereas the effect on oil is variable and appears to depend on a variety of factors. These results add emphasis to the inhibitory effect of the carcinogens on the phospholipid-ascorbic acid system (6). Hydroquinone and similar agents are the only substances shown to inhibit both types of reaction. The antioxygenic activity of hydroquinone, for many chemical reactions, is well known and a considerable literature concerning it has resulted (1). Furthermore, ascorbic acid catalyzes the oxidation of phospholipids but curtails the autoxidation of oils (3, 6). This may be the result of differences in solubility, since ascorbic acid is soluble in the aqueous phospholipid substrate and only very slightly soluble in the oils.

The variable results obtained with the hydrocarbons on different samples of oil might be due to several factors. Each sample of oil varies considerably in both the amount of naturally occurring inhibitors and in the degree of previous oxidation. Variations resulting from differences in age of the oil are also observed. When ethyl linoleate is employed, most of the hydrocarbons stimulate oxidation of fresh preparations but inhibit it in older samples. The oxidation of cod liver oil is inhibited by alloxan while that of corn oil and ethyl linoleate is accelerated. The reason for this is not clear.

SUMMARY

The effect of carcinogenic and related compounds on the autoxidation of certain animal and vegetable oils was investigated. The results were variable and depended upon several factors. It is doubtful whether the observed effects have any influence on the mechanism of cancer formation.

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Induction of Tumors in Rats by Carcinogens in Various Lipids

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The experiments to be described were begun about two years ago, and were prompted by the unsettled status of the effect that different solvents for carcinogens might have on the induction of tumors. Since then, Shimkin and Andervont (14) and Sall and Shear (11) have placed the knowledge of the effects of solvents on a more comprehensive basis. We wish, therefore, to record our experiments on the production of tumors in rats and to indicate why the original objective of showing effects of solvents was not attained.

In 1935 Peacock (9) reported that 1,2,5,6-dibenzanthracene was effective as a tumor-producing agent when dissolved in lard and injected into the breast muscles of fowls, but was ineffective when dissolved in chicken fat. The possibility that the carcinogen might be more readily eliminated when dissolved in species homogenous fat was considered and the next year Chalmers and Peacock (4) studied the elimination of polycyclic hydrocarbons by means of fluorescence. They found that colloidal water suspensions of the hydrocarbons injected intravenously were eliminated by way of the bile, but no specific light was thrown on the question of the fats as solvents. Coincident with their work, Berenblum and Kendall (2) failed to produce any tumors by 30 weekly intravenous injections of colloidal carcinogen in water totalling 20 mgm. of 1,2,5,6-dibenzanthracene given to each of 20 fowls. Later, with mice, Peacock and Beck (10) used 3,4-benzpyrene in powder form, dissolved in ether, in lard, in olive oil alone or olive oil mixed with paraffin, and in mouse lipids. They concluded that the effect of the solvent was that of keeping the carcinogen in place rather than having any effect in itself, and that the powder, ether solution, and mouse fat solution were more readily absorbed and eliminated than the oil solution. Doses of 0.5 to 1.0 mgm. per mouse were used. Oberling and co-workers (8) performed similar experiments with benzpyrene in rats. Olive oil, lard, and rat fat were used for solvents with two levels of dosage, 21 to 25 mgm. per rat in one and 1 mgm. in the other. No appreciable difference in the number and evolution of the tumors produced by the three solutions of each dosage level was observed. Morton and Mider (7) found, however, that 0.25 mgm. of benzpyrene injected subcutaneously into mice gave tumors in only 2 per cent of the animals within 30 weeks when dissolved in mouse fat, but gave tumors in 78 per cent when dissolved in sesame oil and in 50 per cent when used in the colloidal condition.

Twort and Twort (15) painted mice with solutions of dibenzanthracene, benzpyrene, and methylcholanthrene dissolved in chloroform, oleic acid, mineral oil, and liquid paraffin. The chloroform solutions were found to be the most potent. In similar experiments, Crabtree (5) used 0.3 per cent benzpyrene solutions in ether and benzene, both with and without the addition of 2 per cent liquid paraffin. Papillomas appeared 2 to 3 weeks earlier in the mice treated with the solutions which contained the paraffin oil. In a second paper he reported the inhibition of tumor induction by monochloracetal and other chlorinated organic compounds.

Burrows, Hieger, and Kennaway (3) observed the production of a few tumors in rats (but not in mice) by means of lard injections alone. Andervont (1) concluded originally that the solvent medium played no significant role in his tests on 8 different strains of mice, but later, Shimkin and Andervont (14) were able to show that the solvent affected the time of induction when the dose of the carcinogen was near minimal. Observations made by Sall and Shear (11) on the accelerating effect of the basic fraction of creosote oil indicate that a high concentration of carcinogen in the solvent may obscure any effect of substances associated with the solvent itself.

Experimental

Eighty-five white rats of heterogeneous ancestry whose weights ranged between 85 and 290 gm. were used. There were 35 males and 50 females, and these were divided into 10 groups as shown in Table I. All except the 9 rats in group 6 received approximately 8 mgm. of carcinogen administered subcutaneously into the right flank as a warm 2 per cent solution in the different lipids used. A control injection of lipid alone was put into the left flank. The rats of group 6 received 3 injections each, in the right axillary region, lower thoracic region, and flank, 0.25 cc. of a 2 per cent solution of methylcholanthrene in each site with linseed oil, lanolin, and lard respectively as solvents. Similar injections of lipids alone were placed on the left side.

The first six groups received methylcholanthrene.[1] Groups 7-10 received dimethylbenzanthracene.[2] Each rat received one subcutaneous injection except those in group 6, which received three at the same time. The ration was the same for all and consisted of Purina Dog Chow plus weekly supplements of lettuce (or carrots) and beef liver. The lard, lanolin, and sperma-

* Departmental paper No. 347.

[1] Purchased from the Eastman Kodak Co., Rochester, N. Y.

[2] Gift of Dr. W. E. Bachmann.

ceti were melted and allowed to cool to approximately body temperature before injection. A short 22 gauge needle was needed for the spermaceti since it solidified much more quickly than the other two solvents. The linseed oil and rat fat were liquid at room temperature. The rat fat was prepared originally by extracting adipose tissue from the abdominal cavity with acetone, then with petroleum ether. The extracts were combined after evaporation of the solvents, and the whole process carried through at room temperature.

The rats were examined once a week until the tumors began to grow, then twice a week thereafter until sacrificed. The time of the beginning of a tumor was taken as the semi-weekly date immediately preceding the one at which the first certain measurable increment was followed by other progressive increments in size. Fluctuations in size not followed by progressive growth were disregarded.

Results and Discussion

No tumors developed at the site of the injections of lipids alone. These injections appeared to cause a greater tissue reaction than the lipids which contained carcinogen, but the palpable masses disappeared during the first three or four weeks and did not reappear. At injection sites of the lipids which contained carcinogen, palpable masses were slow to appear and did so after about a month. Usually the masses remained as soft to moderately firm 1.0 to 1.5 cm. subcutaneous plaques for several months until progressive growth started. Ulcers did not occur on the control side but occurred occasionally at the site of the injections of methylcholanthrene (less than 10 per cent) and rather frequently (20 to 25 per cent) at the site of the injections of dimethylbenzanthracene. In several instances the formation of an ulcer together with the sloughing out of the area of injection appeared either to delay or prevent tumor formation.

Table I: Time Required for Induction of Malignant Tumors in Rats by Methylcholanthrene and Dimethylbenzanthracene Dissolved in Rat Fat and Other Lipids

Group no.	Original no. rats in group	No. surviving induction period	Solvent	No. tumors	Induction time, days	Average time, days	Remarks
Methylcholanthrene series, groups 1 to 6.							
1	10	9	Rat fat	9	112, 119, 125, 132(2), 153(2), 162(2)	139	
2	6	5	Linseed oil	5	112, 140, 144, 158, 212	153	
3	18	18	Lard	17	117(3), 125,* 125, 130,* 130(4), 133(2), 149, 152, 175, 183, 190	139	One rat killed after 246 days, no tumor.
4	6	6	Lanolin	6	126, 171(2), 175(2), 196	169	
5	4	4	Spermaceti	3	80, 153, 229	154	One rat died after 237 days, no tumor.
6	9	6	Linseed oil, lanolin, and lard	10	131(2), 145(4), 150, 152(2), 175	147	Three injections into each rat. One had 3 tumors, two had 2, and three, 1 each.
Totals.....	53	48		50			
Dimethylbenzanthracene series, groups 7 to 10.							
7	10	7	Rat fat	3	105, 112, 129	115	Four originally had ulcers and died without tumor at 98, 154, 162 and 264 days.
8	6	5	Linseed oil	5	88, 92, 120, 149, 152	120	
9	10	10	Lard	7	88, 98, 108, 131, 160, 202, 287 *	153	Three had ulcers and died without tumors at 108, 113, and 310 days.
10	6	6	Lanolin	6	108, 131, 141, 145, 149, 164	149	
Totals.....	32	28		21			
Grand Totals.	85	76		71			

* Adenocarcinomas of the breast. All other tumors were sarcomas.

In listing the number which survived the induction period, the rats which died before 80 days were not counted, since the first tumor started at that time. Two rats in the methylcholanthrene series and 7 in the dimethylbenzanthracene series died without tumor at intervals from 98 to 310 days after injection. One rat of group 6 (Table I) developed tumors at all three sites of injection, two developed two tumors, and the remaining three succumbed to a single tumor.

There was a nearly complete overlap of the periods of induction in all groups, both with regard to the solvents and carcinogens. There was a trend, however, for the tumors induced by dimethylbenzanthracene to start somewhat sooner than those induced by methylcholanthrene. When the time of occurrence of progressive tumor growth for the two carcinogens is plotted (Fig. 1) a shorter induction period for dimethylbenzanthracene is indicated. A comparison of the averages

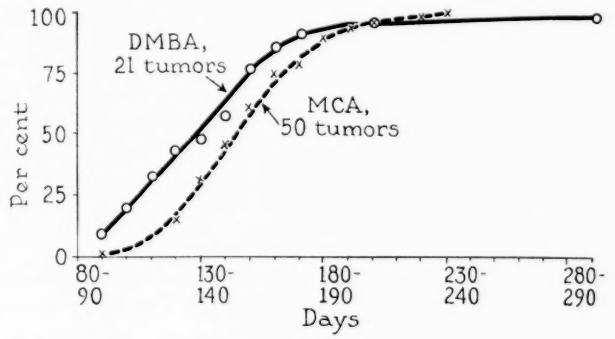

FIG. 1.—Cumulative percentages of tumors originating during 10-day intervals in rats after subcutaneous injection of the carcinogen.
DMBA: 9,10-dimethyl-1,2-benzanthracene, solid line;
MCA: methylcholanthrene, broken line.

of the intervals required for induction is inconclusive with respect to the action of any particular solvent, since the number of animals in each group was too small.

About two-thirds of the total number of tumors were examined histologically and their malignant morphology verified. Three of the 71 tumors were adenocarcinomas of the breast and others were sarcomas. Transplants were made of 51 tumors into other rats. The results on transplantation, together with certain genetic effects seen, will require a separate report since the work is still in progress. Suffice it to say that the percentage of takes with the sarcomas into animals of heterogeneous ancestry was low (approximately 20 per cent). All attempts to transplant the carcinomas failed beyond the first transplant, and very few transient growths were seen in the first passage.

It seems that the amount of carcinogen used, and particularly its concentration in the solvent, has been the chief factor which has been responsible for different results with regard to the influence of the solvent. Morton and Mider (7) used 0.25 mgm. of benzpyrene in a 0.1 per cent solution per mouse. Oberling *et al.* (8) used both large and small doses (21 to 25 mgm. and 1 mgm.) for rats but in solutions of about 2 per cent concentration. The dosage is not stated in the article by Chalmers and Peacock (4), but Peacock and Beck (10) used 0.5 mgm., 0.75 mgm. and 1.0 mgm. per mouse as a 1 per cent solution. Dobrovolskaïa-Zavadskaïa (6) showed that the size of the dose of 1,2,5,6-dibenzanthracene (0.04 per cent in olive oil) affected the incidence of tumor formation, and that female mice were more susceptible than males. Shear and Ilfeld (12) and Shear and Lorenz (13) found that a concentration of at least 1 per cent of carcinogen in cholesterol pellets was necessary to produce tumors with regularity. Shimkin and Andervont (14) found that, in mice, the solvent exerted an effect on both the latent period and the incidence. Methylcholanthrene (0.5 mgm. in 0.25 cc. of solvent) produced tumors in less than half the time when dissolved in tricaprylin than it did when dissolved in butyl phthallate. Differences were noted also among different lots of lard. Sall and Shear (11) have suggested 0.02 mgm. of methylcholanthrene in 0.2 to 0.3 cc. of solvent as a threshold dose for demonstrating the influence of factors in the solvent. In view of these observations, it appears that our dosage of 8 mgm. per rat administered as a 2 per cent solution was too large and too concentrated to reveal any influence of the solvent.

SUMMARY

Methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene produced malignant growths in 80 to 297 days in rats when the carcinogens were dissolved in rat fat or other lipids. Of the 71 tumors produced in an original number of 85 rats, all were sarcomas except three adenocarcinomas of the mammary gland. The dosage of 8 mgm. of carcinogen per rat given in 0.4 cc. of lipid appears to be too large and concentrated to reveal any effects either of the homologous fat or other lipids on the induction time or incidence of tumor formation. Transplantation of the tumors into rats of heterogeneous ancestry gave only 20 per cent progressively growing takes for the sarcomas and none for the carcinomas.

The average induction time for 50 tumors induced by methylcholanthrene was 146.6 days while that for 21 induced by dimethylbenzanthracene was 136.1 days. Rat fat, linseed oil, lard, lanolin, and spermaceti were used as solvents for the carcinogens.

No tumors occurred at the sites of injection of lipids alone.

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Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BERENBLUM, I. [Univ. of Oxford, Oxford, England] THE MECHANISM OF CARCINOGENESIS: A STUDY OF THE SIGNIFICANCE OF COCARCINOGENIC ACTION AND RELATED PHENOMENA. Cancer Research, 1:807-814. 1941.

The effect of croton resin on carcinogenesis was studied under varying conditions. No cocarcinogenic effect was observed when croton resin was applied to the skin and benzpyrene was injected intraperitoneally. Subcutaneous injection of croton resin and benzpyrene together did not augment the carcinogenic effect of benzpyrene. While augmentation of carcinogenesis was pronounced when croton resin was applied to the skin concurrently with a dilute solution of a potent carcinogen (3,4-benzpyrene), none was observed with concentrated solutions of different carcinogens irrespective of whether their potency was high (3,4-benzpyrene), moderate (1,2,5,6-dibenzanthracene), or low (1,2-benzanthracene). Preliminary treatment with croton resin failed to influence the response of the mouse's skin to subsequent application of benzpyrene, but croton resin applied to the skin subsequent to a limited period of benzpyrene treatment led to an increase in the development of tumors. Croton resin applied to papillomas appeared to facilitate their conversion to malignancy.

It is suggested that the 3 phases of carcinogenesis—precarcinogenic, or latent; epicarcinogenic, or wart-stage; and metacarcinogenic, or malignant transformation, are independent processes. Croton resin having only epicarcinogenic and metacarcinogenic properties cannot produce tumors.

Cocarcinogenic action is discussed and attention drawn to the clinical implications of cocarcinogenic, epicarcinogenic, and metacarcinogenic actions on the part of noncarcinogenic agents affecting human beings.—From author's summary.

BRUES, A. M., B. B. MARBLE, and B. RIEGEL. [Collis P. Huntington Memorial Hosp., Boston, Mass. and Northwestern Univ., Evanston, Ill.] FAILURE TO INDUCE SARCOMA IN RATS WITH WHEAT GERM OIL PREPARATIONS. Cancer Research, 1:815-817. 1941.

Thirty-two Wistar and 32 Slonaker albino rats were fed 2.5 to 4.0 cc. daily of four wheat germ oils, including medicinal oil, ether-extracted oil, ether-treated medicinal oil, and ether extract of the wheat germ cake after removal of medicinal oil. These preparations were given for several months. No malignant tumors were found.—Authors' abstract.

DAVENPORT, H. A., J. L. SAVAGE, M. J. DIRSTINE, and F. B. QUEEN. [Northwestern Univ. Med. Sch., Chicago, Ill.] INDUCTION OF TUMORS IN RAT3 BY CARCINOGENS IN VARIOUS LIPIDS. Cancer Research, 1:821-824. 1941.

An attempt was made to detect effects of different solvents used as vehicles for methylcholanthrene and 9,10-dimethyl-1,2-benzanthracene on the induction time of tumor formation in rats. Rat fat, linseed oil, lard, lanolin, and spermaceti were used and a single injection of 0.4 cc. of a 2% solution of the carcinogen made into the right flank. A series of 44 rats received methylcholanthrene and 32 received dimethylbenzanthracene. One special group of 9 received 3 doses of methylcholanthrene in linseed oil, lanolin, and lard respectively. Control doses of lipids without carcinogen were injected on the opposite side. Of the total number of 85 rats, 76 survived longer than 80 days after injection, at which time the first tumor appeared. Between 80 and 287 days, 61 tumors appeared among the 70 which received one injection of carcinogen, and 10 tumors occurred among 6 survivors which received three injections each. The average time of induction in the methylcholanthrene series for each solvent was as follows: rat fat, 139; linseed oil, 153; lard, 139; lanolin, 169; spermaceti, 154; and the 3-injection group, 147 days respectively. The times in the dimethylbenzanthracene series were: rat fat, 115 days; linseed oil, 120; lard, 153; and lanolin, 140. Spermaceti was not used in this series. There was so much overlap in the various times of induction, and the groups so small that the averages are not significant except to indicate that dimethylbenzanthracene is somewhat more active than methylcholanthrene. Any influence of the solvent was probably overwhelmed by the high concentration and large amount of carcinogen used. No tumors developed at the sites of injection of lipids alone.—Authors' abstract.

DEUTSCH, H. F., D. L. MINER, and H. P. RUSCH. [McArdle Memorial Lab., Univ. of Wisconsin, Madison, Wis.] THE EFFECT OF CARCINOGENIC HYDROCARBONS AND RELATED COMPOUNDS ON THE AUTOXIDATION OF OILS. Cancer Research, 1:818-820. 1941.

An effort was made to determine whether the antioxygenic action of the carcinogens is specific for catalyzed phospholipid oxidation or whether other types of fat oxidation are also affected by the hydrocarbons. The effect of various carcinogenic chemicals and related compounds on the autoxidation of corn oil, cod liver oil, lard, and ethyl linoleate was investigated. The degree of autoxidation in the presence or absence of these compounds was determined by the Kreis test, peroxide numbers, and measurement of the total oxygen consumption. When ethyl linoleate was employed, dibenzanthracene acted as a mild inhibitor whereas hydroquinone and ascorbic acid gave marked inhibition. Alloxan, aminoazotoluene, and desoxycholic acid stimulated autoxidation. The effect of other hydrocarbons was variable but, in general, the oxidation of freshly prepared ethyl lineoleate was accelerated by most carcinogens while that of older samples was inhibited. When other oils were employed, the results were even more variable and depended upon several factors.—Authors' abstract.

Hormones

JONES, E. E. [Wellesley Coll., Wellesley, Mass.] THE EFFECT OF TESTOSTERONE PROPIONATE ON MAMMARY

TUMORS IN MICE OF THE C3H STRAIN. Cancer Research, 1:787-789. 1941.

Female mice of the C3H strain were injected with testosterone propionate from the 2nd to the 12th month of life. None developed tumors during the period of treatment. Three (25%) of those living to cancer age developed mammary gland tumors at an average age of 20 months. Eighteen controls (47%) developed tumors at an average age of 12.0 months. Thus, tumor incidence was lowered and age at macroscopic appearance of tumors may have been increased. Mammary glands of treated and virgin control mice of comparable age are described. Glands from treated females had fewer cystic and more narrow branching ducts, more localized areas of alveolar proliferation, and a greater amount of periductal infiltration. Sixteen females of normal breeding history, injected with testosterone, all developed tumors at an average age of 9.6 months. Sib controls all developed tumors at an average age of 9.0 months. Testosterone injections into mice in which a tumor was already established were ineffectual in inhibiting tumor growth.—Author's abstract.

Viruses

BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] THE PRESERVATION BY FREEZING AND DRYING IN VACUO OF THE MILK-INFLUENCE FOR THE DEVELOPMENT OF BREAST CANCER IN MICE. Science, 93:527-528. 1941.

Tissue from spontaneous mammary carcinoma of mice was lyophilized as follows: The tumor tissue was removed aseptically, minced, placed in tubes, immediately frozen at −72° C., and dried *in vacuo*. The tubes were then sealed with a blowtorch till used.

Thirty cc. of water were added to 10 cc. of the lyophilized tumor tissue and filtered. Ten cc. of this filtrate was placed in small dishes before each of 2 groups of 5 mice. The mice receiving this filtrate were of the Ax strain and were from 4 to 5 weeks old. The incidence of breast cancer in breeding females of this strain is 3.1%; average tumor age 13.9 months. Females of this strain are so genetically constituted that breast tumors may be expected to develop if they are nursed by females which have the milk influence, and are used as breeders. Of the mice of the Ax strain receiving filtrate of the lyophilized tumor tissue 6 developed breast tumors (12.4 months), 1 was missing at 12 months of age, and 3 are living (17.5 months). These mice were bred. The 10 experimental animals were the progeny of 3 females, 2 of which had 21 additional young. As none of these mice has developed breast tumors it is improbable that the mice receiving the filtrate were subjected to an active influence in the milk of the mother, or that the influence developed *de novo*. The technic used in the preparation of the lyophilized tumor tissue was similar to that of others for preservation of viruses of other diseases. Hence the results obtained suggest that the milk influence may be a virus.—M. B.

BRYAN, W. R., [Nat. Cancer Inst., Bethesda, Md.] and J. W. BEARD [Duke Univ. Sch. of Med., Durham, N. C.,] STUDIES ON THE PURIFICATION AND PROPERTIES OF THE RABBIT-PAPALLOMA-VIRUS-PROTEIN. J. Nat. Cancer Inst., 1:607-673. 1941.

This large article brings together practically all the known data on the biochemical and infectious properties of the Shope rabbit papilloma virus. Much original work is included. The wealth of material reported is so great and its exposition so detailed that any abstract would be inadequate. The reader is referred to the original paper.—L. L. W.

DURAN-REYNALS, F. [Yale Univ. Sch. of Med., New Haven, Conn.] AGE SUSCEPTIBILITY OF DUCKS TO THE VIRUS OF THE ROUS SARCOMA AND VARIATION OF THE VIRUS IN THE DUCK. Science, 93:501-502. 1941.

The duck, heretofore considered resistant to Rous virus, can be infected if (a) newborn ducks are injected, preferably intravenously; (b) large amounts of virus are used. Intravenous infection of the duckling with the Rous virus is manifest in two ways; by development of a hemorrhagic disease, fatal in a few weeks with blood blebs and extravasations in viscera, and by development of one or a few sarcomas in various locations several weeks or even months after injection. Once tumors have been induced in ducks by the chicken virus, the disease can easily be transmitted by grafts or filtrates to other ducks without much regard for the age of the host.

When the virus has acquired the capacity to infect ducks, it has lost its original capacity to infect chickens. This change seems to take place suddenly as soon as the chicken virus has infected the duck cells, since extracts of hemorrhagic and neoplastic lesions from ducks injected with Rous virus consistently fail to induce tumors in adult chickens. When young chicks (1 to 3 days) are injected intravenously with the duck variant of the Rous virus they develop (in 20 to 40 days) multiple sarcomas of flat and long bones, skeletal muscles, and occasionally in viscera. This disease is quite different from that induced in chicks by the original Rous virus.—M. B.

FRIEDEWALD, W. F. [The Rockefeller Inst. for Med. Research, New York, N. Y.] IDENTITY OF "INHIBITOR" AND ANTIBODY IN EXTRACTS OF VIRUS-INDUCED RABBIT PAPILLOMAS. J. Exper. Med., 72:175-199. 1940.

Virus-induced papillomas of domestic rabbits contain an inhibitor capable of neutralizing the active papilloma virus. Experiments were undertaken to determine whether or not this inhibitor is identical with the specific antiviral antibody which develops in the blood of rabbits carrying papillomas. It was found that the amount of inhibitor present in papilloma extracts varies concurrently with the serum antibody titer of the host. The inhibitor fixed complement in mixture with papilloma virus, in proportion to its neutralizing capacity. It was specifically absorbed from papilloma extracts when mixed with the papilloma virus. The infectivity and complement-fixing property of a virus filtrate could be completely absorbed with the inhibitor, findings similar to those previously reported for blood antibody. The inhibitor and the blood antibody were both completely inactivated by heating 30 minutes at 80°C. and they were precipitated together upon treatment with ammonium sulfate. The inhibitor was not confined to the papillomas but was present in extracts of liver, muscle, and skin from rabbits bearing the papillomas, and in amounts proportional to the titer of antibody in corresponding serums. It is assumed that the greater proportion of inhibitor in papilloma extracts, as compared to organ extracts, is probably blood antibody which has become localized in the papilloma tissues, not merely circulating antibody. This view is supported by

the fact that perfusion with saline failed to effect any large reduction in the inhibitor content of the papillomas. The author concludes that the "inhibitor" demonstrable in extracts of papillomas is identical with the antiviral antibody found in the blood of rabbits bearing the growth.—A. C.

FRIEDEWALD, W. F., and J. G. KIDD. [The Rockefeller Inst. for Med. Research, New York, N. Y.] UNION IN VITRO OF THE PAPILLOMA VIRUS AND ITS ANTIBODY. J. Exper. Med., 72:531-558. 1940.

Observations presented in this paper indicate that the rabbit papilloma virus (Shope) elicits an antibody of one type only, this being capable both of neutralizing the virus and of fixing complement in mixture with it. The virus and its antibody have a specific affinity for one another, each being capable of absorbing the other in great excess when they are brought together in the test tube. These conclusions are based on elaborate experiments which are reported in great detail. The results reveal important properties of the virus and its antibody. General findings can be summarized as follows: 1. The virus-neutralizing and complement-fixing capacities of immune serums, though of widely different origin and potentialities, invariably parallel one another closely. 2. The complement-fixing and virus-neutralizing capacities of any given serum were absorbed simultaneously, the amount of antibody remaining after absorption varying inversely with the concentration of virus used for absorption. 3. Visible flocculation was noted in mixtures of virus filtrate and immune serums when proportions were optimal. In mixtures containing an excess of either, there was less, or no, flocculation. 4. The papilloma virus itself appeared to be responsible for the absorption of antibody, no absorption being observed when extracts derived from domestic rabbit papillomas (inactive), Brown-Pearce tumor, or various mammalian organs, were used. 5. When virus filtrates were fractionated by high speed centrifugation, it was found that absorption occurred only with the fraction containing the virus. 6. In experiments in which the virus had been treated at various temperatures, or had been submitted to ultraviolet light, it was found that infectivity of the virus was affected sooner than the capacity to fix complement or to absorb antibody, but the two latter properties were affected in a comparable manner. The union between the papilloma virus and its antibody *in vitro* appears to be rapid and stable. After 5 minutes contact at room temperature, no evidence of any dissociation could be detected, either by dilution or by centrifugation.—A. C.

Genetics

BITTNER, J. J. [Roscoe B. Jackson Memorial Lab., Bar Harbor, Me.] FOSTER NURSING AND GENETIC SUSCEPTIBILITY FOR TUMORS OF THE BREAST IN MICE. Cancer Research, 1:793-794. 1941.

A high incidence of spontaneous tumors of the breast was observed in breeding females of the A strain and a low incidence in breeding females of the same strain following fostering by mothers of the CBA or X stock (low incidence). Reciprocal matings were made between individuals of these two sublines of the A stock. All the hybrids were used as breeders.

When the maternal parents had high incidences there was no reduction in the F1—F5 hybrids; the reciprocal cross gave only 1 tumor in mice of the F1—F2 generations. The total number of hybrids observed was 859.

Thus, foster nursing, with a reduction in the incidence of tumors, does not alter the genetic constitution of mice for the development of mammary carcinoma.—Author's abstract.

DANZI, M. V., E. BURACK, and A. W. WRIGHT. [Albany Med. Coll., Albany, N. Y.] ABNORMALITIES OF BREEDING BEHAVIOR IN RATS OF THE ALBANY (A-S) STRAIN. Cancer Research, 1:795-798. 1941.

An unusually high rate of infanticide was observed in a strain of rats (Albany or A-S strain) characterized by a high incidence of benign mammary tumors. The cannibalistic habit was manifested too early to be interpreted as an eventual deterioration of the maternal behavior pattern and occurred most frequently in those rats which eventually developed tumors. The data, taken with other observations on breeding abnormalities of these rats, were interpreted as tending to indicate that common factors may be operating in producing both the greater degree of abnormality in reproductive physiology characterizing the tumor-bearing animals and the susceptibility to tumor development.—Authors' abstract.

KIRSCHBAUM, A., and L. C. STRONG. [Yale Univ. Sch. of Med., New Haven, Conn.] TRANSPLANTATION OF LEUKEMIA ARISING IN HYBRID MICE. Cancer Research, 1: 785-786. 1941.

Leukemic cells arising in hybrids (F1, F2, and backcross) between the "susceptible to leukemia" F strain and "resistant to leukemia" CBA strain were inoculated into the parent stocks; leukemias arising in F1 hybrids were also inoculated into F1 hybrid mice. Leukemia arising in hybrid mice was transplantable in the same manner as mammary cancer and normal splenic tissue of hybrid mice; that is, the leukemic cells of F1 hybrids grew in neither parent stock but in all F1 hybrids. Leukemic cells of F2 hybrids grew in neither parent stock; leukemic cells from a small per cent of backcross mice grew in the parent stock to which the F1 hybrid parent had been backcrossed.—Authors' abstract.

MURRAY, W. S. [New York State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] STUDIES ON THE EFFECT OF FOSTER NURSING AND ITS RELATION TO THE DEVELOPMENT OF MAMMARY CARCINOMA IN THE MOUSE. Cancer Research, 1:790-792. 1941.

In this paper an attempt is made to separate and evaluate the parts which chromosomal and extrachromosomal influences play in the development of mammary carcinoma in mice.

Data from 9 experiments totaling 2,230 animals are offered as evidence that the ratios in which mammary tumors occur in inbred strains and experimental crosses may be reasonably explained upon the basis of the strength of the milk stimulus which the mice receive and the resistance of the physiological systems produced by these matings to various concentrations of the milk stimulus.—Author's abstract.

Physical Factors

BURR, H. S. [Yale Univ. Sch. of Med., New Haven, Conn.] CHANGES IN THE FIELD PROPERTIES OF MICE WITH TRANSPLANTED TUMORS. Yale J. Biol. and Med., 13:783-788. 1941.

Significant changes in electrodynamic fields were observed during the growth of transplanted tumors. This supplements previously reported similar observations concerning spontaneous tumors and those induced with methylcholanthrene or benzpyrene.

When the rapidly growing Yale carcinoma No. 1 was implanted into the right axillary region of Strong A mice, the implantation site became significantly positive relative to the other side of the chest at the time of appearance of the tumor. The readings then also exhibited greater variations from the mean than at first. In the sternum-pubis gradient, the former also reversed its polarity and became significantly positive relative to the pubis after the tumor appeared. In the case of the slowly growing tumor No. 139,658A (Strong) the findings were in the same direction. Also in the cross-chest measurements, the values at first clustered about the mean but after the tumor became manifest they tended to scatter. The opposite was observed in the xiphoid-symphysis readings.

These observations concerning gradients are discussed as a frame for a general theory of cancer.—A. A. L.

Radiation

GERSHON-COHEN, J., H. SHAY, and S. S. FELS. [Philadelphia, Penn.] EXPERIMENTAL STUDIES WITH "CONTACT" ROENTGEN RAYS: THE TIME-INTENSITY FACTOR OF THE "TUMOR DOSE" FOR RAT SARCOMA 39 IN SITU. Am. J. Roentgenol., 45:600-604. 1941.

This is a study of massive and fractionated doses of x-ray, delivered by a Chaoul contact therapy on transplanted rat sarcoma 39. The control animals usually died in the 7th week. Spontaneous regression never exceeded 5%. Tumors 10 to 15 mm. in diameter were used. A single dose of 10,000 r caused complete destruction of all tumors. When this total dose was divided and given in daily treatments so that 10,000 r would be delivered within 7, 14, 28, 42, or 70 days, the destructive effect on the tumor decreased. If the total dosage was given in 6 or in 12 successive treatment days, 60% of the animals survived. There was no destructive effect on the tumor if it required 70 days to reach a total dose of 10,000.—E. A. L.

LENZI, M. [Regia Univ., Modena, Italy] A REPORT OF A FEW RECENT EXPERIMENTS ON THE BIOLOGIC EFFECTS OF MAGNETIC FIELDS. Radiology, 35:307-314. 1940.

This is a report of studies on the effect of magnetic fields on cells cultivated *in vitro,* on the repair of cutaneous wounds, and on the growth of Ehrlich adenocarcinoma. The experiments were performed with electromagnets capable of functioning either with direct or pulsating currents and of generating fields of from 1,500 to 1,700 gauss. White mice were used and were placed in cellophane cells between the poles of the magnet.

It was concluded from reviewing the experiments of others that the direct magnetic field acts on the course of mitosis of cells cultivated *in vitro* while the alternating field acts on the order and orientation of the entire culture. Wounds treated in the direct magnetic field showed an initial slowing of the rate of healing compared with the controls followed by an increased rate. This phenomenon has not yet been adequately interpreted.

Grafts of the Ehrlich adenocarcinoma previously kept in a magnetic field show a greater percentage of takes than the control tumor. When the animals were placed in a constant magnetic field, immediately after the graft, for 8 hours a day for 12 days, there was a marked delay in the taking and a smaller percentage of taken neoplasms. With the animals placed in a similar manner in an alternating magnetic field the taking was delayed even more, so that at the end of the 12th day there were 25% takes as compared to 92% in the controls. When animals bearing 12-day-old tumors were used, there was no effect on the further development of the tumors with either type of magnetic field.—E. A. L.

MA, W. C., and CHIEN-LIANG HSU. [Peiping Union Med. Coll., Peiping, China] THE EFFECT OF ROENTGEN RADIATION ON SPINAL GANGLIA OF ALBINO RATS. Am. J. Cancer, 40:335-342. 1940.

The spinal ganglia of rats which had had their extremities irradiated with varying amounts of low voltage x-radiation were studied cytologically. Changes in the cell outlines, the size and shape of the nuclei, and in the Golgi apparatus and mitochondria were noted. Ganglion cells that had received direct radiation showed similar changes. The authors conclude that these changes are morphological evidence of functional disturbances in the cells brought about either directly or by circulating products of irradiation.—L. L. W.

MIWA, M., H. YAMASHITA, and K. MORI. [Japanese Foundation for Cancer Research, Tokyo] THE SPERM OF THE SEA-URCHIN AS A BIOLOGICAL TEST OBJECT IN ROENTGEN DOSIMETRY. Gann, 35:127-132. 1941.

A method for determining the dosimetry of radiation is described which depends on the rate of cleavage of sea-urchin eggs after fertilization by sperm irradiated with varying doses of roentgen rays. These workers found that at a given temperature the time of the first cleavage is determined only by the quantity of radiation administered, and depends neither on the duration of irradiation nor on the time between irradiation and insemination within certain limits. The depth-dose determined by this biological method showed some discrepancy when compared with ionization chamber measurements. The significance of this discrepancy is discussed.—P. P. C.

WARREN, S. [Harvard Med. Sch., Boston, Mass.] THE RADIOSENSITIVITY OF TUMORS. Am. J. Roentgenol., 45:641-650. 1941.

The reactions of neoplasms to radiation fall into three groups: the radiosensitive, which respond strikingly to a total dosage of 2,500 r or less of protracted radiation; radioresponsive, which require from 2,500 to 5,000 r for similar regression; and radioresistant tumors which require over 5,000 r. Radiosensitivity and radiocurability are not, however, synonymous. Although many tumors of higher grades are radioresistant and some of the lower

grades are radiosensitive, as a rule the less differentiated tumors are radiosensitive.

Chronic myelogenous leukemias and the lymphomas are examples of the radiosensitive tumors. Rare apparent cures may be obtained. Examples of the radioresponsive tumors are the basal cell carcinoma of the skin and carcinoma of the cervix uteri. Carcinoma of the breast and of the gastrointestinal tract and malignant melanomas are radioresistant.—E. A. L.

ZAHL, P. A., F. S. COOPER, and J. R. DUNNING. [Columbia Univ., New York, N. Y.] SOME IN VIVO EFFECTS OF LOCALIZED NUCLEAR DISINTEGRATION PRODUCTS OF A TRANSPLANTABLE MOUSE SARCOMA. Proc. Nat. Acad. Sc., 26:590-598. 1940.

Transplantable mouse sarcomas were injected with various forms of slow neutron-capturing materials. When the whole animal whose tumor was so injected was irradiated with slow neutrons, a significant increase in tumor regression was observed. This increase is attributed to the localized ionization resulting from the nuclear disintegration products of the capture process.—Authors' summary.

Biochemistry and Nutrition—Chemotherapy

FIGGE, F. H. J., and L. C. STRONG. [Yale Univ. Sch. of Med., New Haven, Conn., and Univ. of Maryland Sch. of Med., Baltimore, Md.] XANTHINE OXIDASE ACTIVITY IN LIVERS OF MICE OF CANCER-SUSCEPTIBLE AND CANCER-RESISTANT STRAINS. Cancer Research, 1:779-784. 1941.

Xanthine oxidase occurs abundantly in liver, mammary glands, and raw milk. This enzyme oxidizes aldehydes as well as some of the constituents of nucleoproteins and, in so doing, maintains very negative oxidation reduction potentials. A possible relationship to previous work on salicylaldehyde tolerance and heptaldehyde influence on tumors was, therefore, postulated.

A method for determining the xanthine oxidase activity of mouse livers, utilizing the Thunberg methylene blue reduction technic, was devised. The essential and new feature of this method was the removal of naturally occurring substrates and end products by dialysis. Xanthine oxidase activity was determined quantitatively in livers of 21 mice of the C3H cancer-susceptible strain and 22 mice of the JK cancer-resistant strain. The C3H livers averaged 0.81 xanthine oxidase activity units per gm. of liver. The JK livers averaged 1.64 xanthine oxidase activity units per gm. of liver. These values correspond to a methylene blue reduction time of 33 minutes per C3H and 16 minutes for JK livers.

It is thus apparent that the xanthine oxidase activity in livers of the mammary cancer-resistant JK strain of mice is about double that observed in the livers of C3H mammary cancer-susceptible strain of mice. A discussion of a possible correlation between xanthine oxidase activity and cancer susceptibility is included.—Authors' abstract.

LASNITZKI, A., and A. K. BREWER. [Univ. of Manchester, Manchester, England, and U. S. Dept. of Agric., Washington, D. C.] THE ISOTOPIC CONSTITUTION OF POTASSIUM IN ANIMAL TUMORS AND MUSCLE FROM TUMOR-BEARING ANIMALS. Cancer Research, 1:776-778. 1941.

The isotopic constitution of potassium in Jensen rat sarcoma and mouse sarcoma S 37 has been studied. Compared to mineral potassium as contained in ordinary potassium chloride (A. R.), a slight but definite increase of the isotopic ratio K^{39}/K^{41} was found, indicating a corresponding decrease in the percentage of the heavy isotope K^{41}. In rats, since potassium in bone including marrow and blood plasma has shown similar deviation in the opposite direction, and as potassium in other normal tissues usually showed no deviation, it appears that the isotopic constitution of potassium in normal and tumor tissue is appreciably different. Comparison was also made of the potassium in muscle from normal and tumor-bearing rats and mice. In muscle from normal animals the isotopic ratio was generally the same as that of mineral potassium; but in tumor-bearing animals a deviation was observed similar to that found with tumor tissue.—Authors' summary.

LEWISOHN, R., C. LEUCHTENBERGER, R. LEUCHTENBERGER, D. LAZLO, and K. BLOCH. [The Mount Sinai Hosp., New York, N. Y.] ACTION OF YEAST EXTRACT ON TRANSPLANTED AND SPONTANEOUS MALIGNANT TUMORS IN MICE. Cancer Research, 1:799-806. 1941.

In testing the action of fractions of yeast extract on the regression of spontaneous adenocarcinoma of the mouse, the following results were obtained. The active principle is water-soluble and comparatively thermostable at neutral pH. It is not protein in nature, not affected by nitrous acid, nor precipitated by high concentration of ethanol. The active material is precipitated by lead acetate and silver nitrate. Active preparations can be obtained by precipitation with barium and ethanol, and also by phosphotungstic acid. The active material is adsorbed by Fuller's earth and by norite but not by permutit. It has not been possible to remove it by elution. None of the known vitamins of the B group appears to be responsible for the activity.

Results with yeast extract on four different malignant tumors in mice are reported. These were spontaneous mammary adenocarcinomas in mice of the following strains: 1. strain A, Jackson Memorial Laboratory; 2. Rockland Farms strain; 3. strain R III. One tumor was the highly malignant transplanted carcinoma 2163 in the R III strain. With all these tumors 30% complete disappearance was produced.

In the spontaneous carcinoma (R III strain) results obtained in biopsied and nonbiopsied tumors were found to be identical. As a rule small tumors respond to the yeast extract more quickly than large tumors. Recurrences are noted in about 25% of the apparently healed animals, usually after 6 to 8 months.

Fifty spontaneous mammary carcinomas are presented. Biopsy performed before treatment was started had established the diagnosis of carcinoma. Intravenous treatment either with spleen or yeast extract was given. The tumors had disappeared completely for 1 to 12 months when the animals died. Careful post-mortem and microscopical examination failed to show any evidence of remaining tumor cells. Twenty animals are still living and apparently healed. Among the 70 healed animals, 50 belonged to the strain A Jackson Memorial Laboratory and 20 to the Rockland Farms strain.

The interpretation of the regressive changes in the tumors of the treated animals is difficult, as similar changes,

though not as extensive, may be observed in untreated tumors. However, in successfully treated animals the whole tumor undergoes marked changes in a relatively short time, whereas in the controls these changes occur more slowly. Remnants of unchanged malignant tumor tissue are always present in the controls. Histological pictures of marked changes in treated tumors (as compared with the original biopsy specimen) after 7 and 43 intravenous injections respectively are presented.—Authors' abstract.

MORI, K. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] EFFECT OF ANIMAL TISSUE FEEDING ON EXPERIMENTAL PRODUCTION OF LIVER CANCER, ESPECIALLY THE INHIBITING EFFECT OF KIDNEY FEEDING. Gann, 35:86-104. 1941.

In continuation of previous experiments which showed that liver feeding prevented carcinogenesis by butter yellow, the author investigated the effect of the following tissues: beef kidney, spleen, muscle, brain, lung, fore and glandular stomachs, small intestine, pancreas, and testicle. In addition, the effect of feeding bile was investigated. The different tissues were dried on a steam bath and pulverized. The diets consisted of 880 gm. of polished rice, 100 gm. of dried tissue, and 20 gm. of an olive oil solution of butter yellow. The amount of butter yellow used was 0.2 gm. per kg. of food at the beginning of the experiments, and was gradually increased to 0.6 gm. per kg. A small slice of carrot was fed every other day. The experiments were terminated after 150 days and the livers were examined macroscopically and microscopically. Forty to 50 animals were used for each experiment. Of the above tissues kidney alone showed an inhibitory effect on carcinogenesis, though the effect was not as marked as with liver feeding. In the case of the bile feeding experiments, the mortality was so high that no rats survived beyond 131 days, and as many as 31 of 40 animals died during the early part of the experiment. Thus of the animal tissues, liver and kidney are the only ones which exert an inhibitory influence on butter yellow carcinogenesis.—P. P. C.

MORI, K. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] EFFECT OF LIVER FEEDING ON LIVER CANCER PRODUCTION BY o-AMINOAZOTOLUOL. Gann, 35:106-120. 1941.

One hundred rats were placed on a diet consisting of polished rice (900 gm.), o-aminoazotoluol (0.2 to 1 gm. per kg. of food) in olive oil, dried beef liver powder (100 gm.), and fresh carrots. A second group of 100 rats was placed on the same diet except that no liver powder was present. The experiment was discontinued after 350 days at which time 38 rats on the liver diet and 24 rats on the nonliver control diet survived. In the liver fed group, 23 (60.5%) of the total 38 rats showed apparently normal livers. In 13 other animals (34.2%) the liver changes were not advanced beyond the stage of a granular surface, and only in the remaining 2 animals (5.2%) were the hepatic changes sufficiently advanced to warrant the diagnosis of cancer, but without cirrhosis. In contrast, the group not fed liver included 8 animals (33.3%) with liver cancer plus cirrhosis and 16 animals (66.6%) with typical cirrhosis. None of the control rats showed normal livers macroscopically.—P. P. C.

MORI, K. [Labs. of the Japanese Foundation for Cancer Research, Tokyo] ON THE EFFECT OF CYSTINE FEEDING ON EXPERIMENTAL PRODUCTION OF LIVER CANCER. Gann, 35:121-126. 1941.

Forty rats were fed a diet consisting of polished rice, butter yellow (0.2-0.6% of diet in olive oil), cystine (1 gm. per kg. of polished rice), plus a daily slice of carrot. An equal number of animals were fed the above diet without added cystine. The animals were sacrificed after 150 days. In the cystine-fed group 16 rats survived, 6 (37.5%) of which showed cirrhosis, and the remaining 10 (62.5%) liver cancer. In the control group 8 animals survived, 3 (37.5%) of which showed cirrhosis and 5 (62.5%) of which showed liver cancer. The author concludes that on the basis of this study the inhibiting effect of liver on butter yellow carcinogenesis cannot be due to the high sulfhydryl content of these tissues.—P. P. C.

MORIGAMI, S., and N. KASIWABARA. [Osaka Imperial Univ.] INHIBITION OF THE EXPERIMENTAL PRODUCTION OF LIVER CANCER BY MILLET FEEDING. Gann, 35:65-70. 1941.

Fifty rats were placed on a diet of husked and polished rice, 20 cc. of 3% butter yellow in olive oil, green vegetables, and dried sardines. An equal number of animals were placed on the same diet excepting that husked Japanese millet was used in place of rice. All the livers of the animals which died were carefully examined histologically. At the end of 165 days all the surviving animals, 5 in the rice group and 10 in the millet group, were sacrificed for histological examination. Liver cancer was found in 100% of the animals surviving in the rice group. No cancer was found in the 10 animals surviving on the millet diet. The livers of the latter animals showed minimal pathological changes.—P. P. C.

ORR, J. W., and L. H. STICKLAND. [Univ. of Leeds, Leeds, England] THE METABOLISM OF RAT LIVER DURING CARCINOGENESIS BY BUTTER YELLOW. Biochem. J., 35:479-487. 1941.

An examination of Nakatani's statement that during carcinogenesis by butter yellow (dimethyl yellow, p-dimethylaminoazobenzene) the glycolytic properties of normal liver tissue change progressively to those of liver tumor tissue. The steps in the process were classified histologically, (normal, degenerative changes, periportal changes, nodular hyperplasia). Anaerobic glycolysis, $Q^{N_2}_{CO_2}$, stated by Warburg and Nakatani to be low in normal liver was found to depend in some degree upon the glycogen content. When comparison is made giving due regard to glycogen content, no significant difference was seen between $Q^{N_2}_{CO_2}$ for normal, regenerating or precancerous liver. $Q^{N_2}_{CO_2}$, as a measure of the capacity to deal with added glucose (*i.e.* glucolysis) was small in amount and there was no great difference in this respect between normal, regenerating, or precancerous liver. Normal liver has also a very small aerobic glucolysis; *i.e.,* conversion of added glucose to lactic acid aerobically. Liver tumors have quite different glycolytic characteristics. Two hepatomas had high anaerobic glucolysis, and 3 cholangiomas, and 2 hepatomas had considerable aerobic glucolysis (about $\frac{1}{3}$ as high as the anaerobic glucolysis). Thus the change in metabolism during carcinogenesis seems to be sudden

and not progressive and the substrate is glycogen in liver tissue and glucose in liver tumors.

The authors point out that comparison of the metabolism of a parent tissue and of the tumors derived from it has been investigated only for liver and for skin (Berenblum *et al.*,) who found no qualitative differences between normal skin and skin tumors.—I. H.

POPPER, H., and A. B. RAGINS. [Cook County Hosp., Chicago, Ill.] HISTOLOGIC DEMONSTRATION OF VITAMIN A IN TUMORS. Arch. Path., 32:258-271. 1941.

With the fluorescence microscope 219 tumors were examined for vitamin A fluorescence, which was encountered only in certain groups of tumors. The findings suggest that the presence or absence of vitamin A does not influence tumor formation. Vitamin A fluorescence is seen in tumors originating from a parent tissue normally containing vitamin A. The presence and distribution of vitamin A fluorescence helps to determine the origin of some tumors; *e.g.*, ovarian tumors. The fluorescence microscopic picture suggests an adrenal origin of hypernephroma. The visualization of vitamin A in tumors further suggests an important histologic method for the study of the histogenesis of tumors.—Authors' summary.

WHITE, A. [Yale Univ. Sch. of Med., New Haven, Conn.] GROWTH-INHIBITION PRODUCED IN RATS BY THE ORAL ADMINISTRATION OF SODIUM BENZOATE. EFFECTS OF VARIOUS DIETARY SUPPLEMENTS. Yale J. Biol. & Med., 13:759-768. 1941.

Previous experiments concerning the effect of sulfur-containing amino acids, and of glutathione and cystine disulfoxide in terminating the growth inhibition produced by certain compounds is reviewed. The question had arisen as to whether a chemical detoxicating mechanism or one of general growth stimulation is concerned. In the present experiments the introduction of 5% of sodium benzoate into a low protein diet was found to inhibit the growth of young rats. This inhibition of growth was abolished by adding to the diet glycine, sarcosine, or glycolic acid (in optimal quantity); there is evidence that both sarcosine and, to some extent, glycolic acid can be converted to glycine. Ineffective, however, were the sulfur-containing amino acids, and glycocyamine, creatine, hydroxyproline, serine, threonine, sodium sulfate, or sodium citrate.

These results were taken as evidence that a detoxicating reaction is concerned in the growth inhibition: the effective dietary supplement supplies for conjugation a substance that otherwise would be withdrawn from the tissues of the animal subsisting on a low protein diet.—A. A. L.

Immunology

PARKER, R. F., and L. H. BRONSON. [Western Reserve Univ. Sch. of Med. and Lakeside Hosp., Cleveland, Ohio] NEUTRALIZATION OF VIRUS OF MYXOMA BY SPECIFIC IMMUNE SERUM. J. Immunol., 40:147-152. 1941.

The serum of rabbits convalescent from infectious myxoma possessed the power to neutralize the virus of the disease.—M. J. E.

Cytology

WILLIAMS, W. L., K. F. STEIN, and E. ALLEN. [Yale Univ. Sch. of Med., New Haven, Conn., and Mount Holyoke Coll., South Hadley, Mass.] REACTION OF GENITAL TISSUES OF THE FEMALE MOUSE TO THE LOCAL APPLICATION OF COLCHICINE. Yale J. Biol. & Med., 13:841-846. 1941.

Upon direct contact with the genital tissues of spayed and subsequently estrogenized mice, colchicine (0.1 mgm. in 0.05 cc. of H_2O) produced surface and systemic effects. Local action upon the vaginal epithelium was demonstrated within half an hour after contact with the drug, reached a maximum between the 6th and 8th hour, was on the decline at the 10th, and was largely absent at 24 hours. After the 6th hour colchicine effect was visible also in the uterus and rectum; this indicated systemic action. No effect was observed in mice spayed but not treated with estrone. Colchicine introduced in similar amount into a ligated uterine horn produced effects only on the homolateral horn. In the periovarian capsule colchicine action was local; it was manifest only in the 6 to 8 peripheral layers of cells and in the outer portions of the larger follicles.—A. A. L.

Miscellaneous

ABRAMSON, H. A., and M. H. GORIN. [Biological Lab., Cold Spring Harbor, Long Island, N. Y. Labs. of the Mount Sinai Hosp., New York, N. Y.] SKIN REACTIONS. IX. THE ELECTROPHORETIC DEMONSTRATION OF THE PATENT PORES OF THE LIVING HUMAN SKIN; ITS RELATION TO THE CHARGE OF THE SKIN. J. Phys. Chem., 44:1094-1102. 1940.

Active agents of dialyzed extracts of giant ragweed, short ragweed, and timothy pollen can be transported by diffusion and electrophoresis into the skin. Three main channels of transport are considered, (a) the pores or coils of the sweat glands, (b) the hair follicles and sebaceous glands, (c) the keratin matrix of the skin itself. Electrophoretic studies show that copper ions, and basic and acidic dyestuffs localize primarily in the sweat glands, and that pore patterns of the skin, persisting for several weeks may be "developed" by electrophoretic transport of such pigments. Pore patterns in scar tissue and in such skin diseases as scleroderma and psoriasis may also be mapped in this manner and certain characteristics of the disease defined. Although not specifically mentioned by the authors, it would seem that such a technic might be useful in studies of cancerous skin lesions.—R. N. J.

PEACOCK, P. R. [Glasgow Roy. Cancer Hosp., Glasgow, Scotland] CHOLECYSTOSTOMY FISTULAE SUITABLE FOR SMALL LABORATORY ANIMALS. Quart. J. Exper. Physiol., 30:303-311. 1941.

An improved form of an operation devised for the study of the metabolism of carcinogenic compounds.—E. L. K.

RONDONI, P. [Inst. für exper. Path. der Univ. und Krebsinst., Milan, Italy] DAS NEOPHELOMETRISCHE VERHALTEN VON SERUM UND VON ORGAN- UND TUMOREIWEISS BEI DER ERWÄRMUNG. [NEPHELOMETRIC REACTION OF SERUM, ORGAN, AND TUMOR PROTEIN TO HEAT. Ztschr. f. Immunitätsforsch., 99:110-121. 1940.

Variations in the Tyndall phenomenon observed in serums, and extracts of normal and neoplastic tissues at different temperatures were studied with the aid of a nephelometer. On heating rabbit and horse serum there is a relative decrease in the Tyndall effect between 44° and 62° C. prior to the rapid flocculation of the protein

molecules at the latter temperature. A 10% sodium chloride extract of the normal subcutaneous tissue of the rat reacted somewhat similarly although the relative increase in clarity was restricted to a zone between 45° and 55° C. This biphasic reaction was absent in comparable concentrations of protein of induced benzpyrene or methylcholanthrene sarcoma of the rat. Further, in tumor extracts an increase in Tyndall effect was noted at a temperature of 46-51° C., thus indicating the presence of more labile proteins. In more dilute tumor protein suspensions a slight relative increase in clarification occurred at lower temperatures. Rabbit liver and kidney proteins reacted similarly to tumor extracts.—M. J. E.

VADÁSZ, J. [Biol. Forschungstation, Alsógöd, Hungary] ÜBER DIE PHAGOZYTOSE DER KREBSZELLEN UND DIE IN DEN KREBSZELLEN BEFINDLICHEN EINSCHLUSSKÖRPERCHEN. [PHAGOCYTOSIS BY CANCER CELLS AND INTRACELLULAR INCLUSION BODIES.] Arch. f. exper. Zellforsch., 24:137-140. 1940.

With the aid of a microcinematographic apparatus active phagocytosis of leucocytes and wandering cells by tumor cells was observed in explants of a mouse carcinoma. At a later stage remnants of the phagocytosed cells are frequently encountered as inclusion bodies in the cytoplasm of the malignant cells.—M. J. E.

COMPARATIVE ONCOLOGY

LIPSCHÜTZ, A. [Nat. Health Service, Republic of Chile, Santiago, Chile] SPONTANEOUS FIBROMYOMA IN THE FEMALE GUINEA PIG. Arch. Path., 31:702-705. 1941.

Uterine fibroids can be induced in the guinea pig by prolonged treatment with estrogens, but spontaneous fibroids are extremely rare. One small pedunculated uterine fibromyoma was found in a group of 94 untreated, noncastrated adult females. Its microscopic structure was similar to that of fibromyoma in woman and different from that of experimental fibroid in the guinea pig.—H. G. W.

PAPANICOLAOU, G. N., and C. T. OLCOTT. [Cornell Univ. Med. Coll., New York, N. Y.] STUDIES OF SPONTANEOUS TUMORS IN GUINEA-PIGS. I. A FIBROMYOMA OF THE STOMACH WITH ADENOMA (FOCAL HYPERPLASIA) OF THE RIGHT ADRENAL. Am. J. Cancer, 40:310-320. 1940.

In a 7-year-old female guinea pig of unknown ancestry, 2 tumors were found at autopsy, one a pedunculated fibromyoma arising subserosally from the pyloric stomach, the other an adenoma of the adrenal. The animal had shown some irregularities in its estrous cycles before death. Careful examination of the other endocrine glands revealed only senile changes.—L. L. W.

Clinical and Pathological Reports

Heredity

MACKLIN, M. T. [Univ. of Western Ontario Med. Sch., London, Ont.] TUMOURS IN MONOZYGOUS AND DIZYGOUS TWINS. (A REPORT OF NINETEEN NEW CASES.) Canad. M. A. J., 44:604-606. 1941.

Of 9 pairs of monozygous twins in whom neoplastic disease occurred in one or both members, both were affected in 6 and one in 3 instances, while of 10 pairs of dizygous twins both were affected in only 2 and one in 8 instances. Tumors occurring in twins were frequently similar in each member of a pair.—M. J. E.

WHITE, J. W. [St. Mary's Hosp., Scranton, Pa.] MULTIPLE PRIMARY MALIGNANT TUMORS. Am. J. Surg., 53: 71-81. 1941.

A review of the literature discloses an incidence of primary multiplicity of between 3.5 and 4% of all malignant lesions. Report of a case of simultaneous occurrence of cancer of the face and the breast, and another of carcinoma of the lip and the thyroid. The hypothesis is advanced that the genesis of multiple cancers depends on the interaction of a functionally mature gene bearing the unit character for cancer inheritance with a functionally mature gene bearing the unit character for localization but endowed with the faculty to affect various structures similarly.—H. G. W.

Diagnosis—General

MENDES FERREIRA, A. E. [Mayo Clinic, Rochester, Minn.] RELATION OF THE MACRONUCLEOLUS AND NUCLEONUCLEOLAR RATIO TO HISTOLOGIC GRADING. J. Lab. & Clin. Med., 26:1612-1628. 1941.

The average areas of the nuclei and nucleoli, and the average nucleonucleolar ratio, are not the same in all normal tissues; they vary, probably according to the function of the organ, and in the same organ they vary with the metabolic activity. In nonmalignant lesions the average area of the nucleoli is smaller and the average nucleonucleolar ratio is larger than in carcinoma. In the cells of the fetus, normal liver, membrana granulosa of the ovary, and exophthalmic goiter the areas of nucleoli and the nucleonucleolar ratios approximate those found in carcinoma. This is probably attributable to the great metabolic activity of these cells. There is more variation in the extreme areas of the nuclei and nucleoli in carcinoma than there is in nonmalignant lesions. The average area of the nucleoli and the average nucleonucleolar ratio vary according to the degree of malignancy; the former in a direct and the latter in an inverse proportion. In carcinoma the average area of the nucleoli increases and the average nucleonucleolar ratio decreases from grade 1 to grade 2, from grade 2 to 3, and from 3 to grade 4. The increased amount of nucleolar substance in cancer cells is most probably attributable to the great cellular metabolism and cellular division characteristic of those cells. The increasing of nucleolar substance associated with the increasing in the grade of carcinoma is most probably attributable to the increase of cellular metabolism and cellular division from grade 1 to 2, from grade 2 to 3, and from grade 3 to 4.—Author's summary.

MEYER, R. [Univ. of Minnesota, Minneapolis, Minn.] THE BASIS OF THE HISTOLOGICAL DIAGNOSIS OF CARCINOMA. Surg. Gynec. & Obst., 73:14-20. 1941.

A lecture on the differentiation of carcinoma of the cervix uteri and reparative lesions.—H. G. W.

STERN, K. [New York Univ. Med. Coll., New York, N. Y.] ADSORPTION OF SURFACE-ACTIVE SUBSTANCES OF

URINES, WITH SPECIAL REFERENCE TO MALIGNANT NEOPLASIA. Am. J. M. Sc., 202:229-237. 1941.

Studies on the surface tension and on the adsorption of the surface-active substance were performed on 118 urines, from normal persons and from patients suffering from various diseases. In the majority of the urines of patients afflicted with malignant neoplasms, acute infections, or tuberculosis, a characteristic quality of the urinary surface-active substances was observed with regard to their adsorbability.—H. G. W.

Nervous System

PENFIELD, W., T. C. ERICKSON, and I. TARLOV. [Montreal Neurol. Inst. and McGill Univ. Montreal, Canada] RELATION OF INTRACRANIAL TUMORS AND SYMPTOMATIC EPILEPSY. Arch. Neurol. & Psychiat., 44:300-315. 1940.

The occurrence of focal epileptic seizures in 703 cases of intracranial tumor is discussed.—M. J. E.

RAY, B. S., and N. C. FOOT. [New York Hosp. and Cornell Univ. Med. Coll., New York, N. Y.] PRIMARY MELANOTIC TUMORS OF THE MENINGES: RESEMBLANCE TO MENINGIOMAS. REPORT OF TWO CASES IN WHICH OPERATION WAS PERFORMED. Arch. Neurol. & Psychiat., 44:104-117. 1940.

Localized, deeply melanotic subdural tumors were excised from the lumbar cord and the occipital lobe respectively. The growths did not invade the substance of the spinal cord or brain, but in the second case the dura about the tumor was pigmented and neoplastic cells had extended into the covering occipital bone. From the standpoint of structure and effects on the patients the tumors closely resembled meningioma. Their only relationship to melanoma was the presence of specific pigment granules. The postoperative course was favorable and the first patient appeared tumor-free after 5 years, the second after 2 years. Roentgen therapy was administered in the second case to the exposed brain after removal of the tumor nodule (2000 r) and postoperatively (4,200 r). Drawings of the lesions *in situ* and photomicrographs are reproduced.—M. J. E.

SCHEINKER, I. [Clin. Neurol. Hospice de la Salpêtrière, Paris, France] CEREBRAL SWELLING AND EDEMA ASSOCIATED WITH CEREBRAL TUMOR. A HISTOGENETIC AND HISTOPATHOLOGIC STUDY. Arch. Neurol. & Psychiat., 45:117-129. 1941.

The author distinguishes cerebral swelling and edema as graded but basically similar reactions of the brain tissue about and at a distance from cerebral neoplasms. Ten cases of benign and malignant tumor form the basis of the report. Photographs of gross specimens and photomicrographs illustrate the report.—M. J. E.

WALKER, A. E. [Univ. of Chicago, Chicago, Ill.] EARLY DIAGNOSIS OF SPINAL CORD TUMOR. J. Indiana M. A., 33:360-366. 1940.

A general outline of the symptoms and findings in cases of spinal cord tumor is given. The paucity and chronicity of the early symptoms, and their indefinite nature leading frequently to erroneous diagnoses of rheumatism, back strain, neuritis, or gall bladder disease are stressed. Incorrect treatment is consequently applied. The true nature of the lesion is generally not established until paresis, paralysis, sensory loss, and deficient bladder control are fully developed. Photographs of tumors *in situ* are reproduced.—M. J. E.

WALKER, A. E. [Univ. of Chicago, Chicago, Ill.] ASTROCYTOSIS ARACHNOIDEAE CEREBELLI: A RARE MANIFESTATION OF VON RECKLINGHAUSEN'S NEUROFIBROMATOSIS. Arch. Neurol. and Psychiat., 45:520-532. 1941.

Post-mortem examination on a 16-year-old girl with long-standing manifestations of cerebellar disease and neurofibromas of the thoracic and abdominal sympathetic nerves disclosed extensive tumor-like proliferation of astrocytes of the meninges covering the right cerebellar hemisphere with extension of the process to the fourth ventricle.—M. J. E.

WEINBERGER, L. M., and F. C. GRANT. [Univ. of Pennsylvania Hosp., Philadelphia, Penn.] PRECOCIOUS PUBERTY AND TUMORS OF THE HYPOTHALAMUS. Arch. Int. Med., 67:762-792. 1941.

A case is reported which supports the concept that the supposed effects of pineal tumors in causing sexual precocity really depend on involvement of the hypothalamus, with uncontrolled release of pituitary substance, rather than to overproduction of any hypothetic and as yet unproved pineal secretion.—H. G. W.

WEINBERGER, L. M., and J. E. WEBSTER. [Hosp. of Univ. of Pennsylvania, Philadelphia, Penn.] VISUAL FIELD DEFECTS ASSOCIATED WITH CEREBELLAR TUMORS. Arch. Ophth., 25:128-138. 1941.

Defects in vision accompanying intracranial tumors generally arise from direct compression of the optic pathways. They are not considered characteristic of cerebellar tumors and if observed in a case with other evidence of a cerebellar location of an expanding focus, are likely to cause confusion in diagnosis. Eight cases of cerebellar neoplasm are recorded in which visual field defects occurred, and in 7 it was necessary to resort to ventriculography to ascertain the diagnosis with certainty. Four patients had partial or complete homonymous hemianopia, 1 a bitemporal hemianopia, and 3 irregular atypical field defects. The disturbances in the optic system are the result of obstruction of the cerebral aqueduct by the cerebellar tumor with resultant ventricular dilatation and variable types of compression of the optic chiasm by the distended third ventricle.—M. J. E.

WEINSTEIN, E. A., and I. S. WECHSLER. [Mt. Sinai Hosp., New York, N. Y.] DERMOID TUMOR IN THE FORAMEN MAGNUM, WITH ASTEREOGNOSIS AND DISSOCIATED SENSORY LOSS. Arch. Neurol. & Psychiat., 44: 162-174. 1940.

An attempt was made to remove surgically a portion of a dermoid attached to the medulla. The patient died postoperatively. Extensive degenerative changes were present in the medulla as a result of compression by the growth. A photograph of the brain and photomicrographs are reproduced.—M. J. E.

Eye

ALBERS, E. S. [Christie Clinic, Champaign, Ill.] BENIGN MELANOMAS OF CHOROID AND THEIR MALIGNANT TRANSFORMATION. Am. J. Ophth., 23:779-783. 1940.

Benign melanomas of the choroid are considered analagous to pigmented nevi of the skin. They are visible ophthalmoscopically as localized slate gray spots in the fundus. They may undergo malignant transformation, as is demonstrated by the author's case. A benign lesion had been observed in a woman of 60 years 8 years prior

to the present illness. She now had a large intra-ocular malignant melanoma completely obliterating vision in the affected eye. The eye was enucleated, but the patient died of hepatic metastases 2 years later.—M. J. E.

GIVNER, I. [New York, N. Y.] BENIGN MELANOMA OF THE CILIARY BODY. Arch. Ophth., 24:347-351. 1940.

A localized small benign melanoma (pigmented nevus) of the ciliary body was an incidental necropsy finding in a woman of 63 years who died of arteriosclerotic heart disease.—M. J. E.

SONDERS, B. F. [Hosp. of Univ. of Pennsylvania, Philadelphia, Penn.] TRANSCRANIAL EXTIRPATION OF A FIBROHEMANGIOMA OF THE ORBIT. Arch. Ophth., 24: 539-543. 1940.

A fibrohemangioma of the posterior orbit, which had produced exophthalmos of 6 years' duration, was successfully excised through a transfrontal incision and removal of the orbital roof. The exophthalmos regressed completely after operation and there was excellent retention of function in the eye of the previously involved side.—M. J. E.

SPRATT, C. N. [Minneapolis, Minn.] CARCINOMA OF THE LACRIMAL SAC. REPORT OF A SECOND CASE. Arch. Ophth., 24:1237-1243. 1940.

Resection of the orbital tissues was successfully performed for an epidermoid carcinoma arising in the lacrimal sac. Postoperative roentgen therapy (200 kv., 5,400 r in fractionated doses) was administered and the patient appeared tumor-free 10 months later.—M. J. E.

TERRY, T. L. [Boston, Mass.] MALIGNANT MELANOMA—SO-CALLED SARCOMA—OF UVEA. III. EXTENSION INTO THE OPTIC NERVE. Arch. Ophth., 24:206-214. 1940.

Two cases are recorded of extension of intraocular melanoma to the optic nerve and interior of the skull. In the first patient the tumor arose in the iris and extended into the ciliary body, retina, and optic nerve by way of the lamina cribrosa. In the second the growth was situated in the posterior portion of the choroid and invaded massively the retina and optic nerve. Enucleation was performed in both cases and in the second, in addition, an exenteration of the orbit was resorted to 4 months after the first operation. Both patients died of intracranial melanoma deposits originating in optic nerve extension of the tumor, the first 7 years after operation, and the second after 28 months. The author suggests that suspicious optic nerve extension of melanoma in a patient who is a good operative risk requires immediate radical treatment, consisting not only of exenteration of the orbital tissues, but also of intracranial and interosseous removal of the optic nerve.—M. J. E.

von SALLMANN, L. [Herman Knapp Memorial Eye Hosp., New York, N. Y.] GELATINOUS CANCER OF THE CHOROID FOLLOWING CARCINOMA OF THE RECTUM. Arch. Ophth., 25:89-92. 1941.

Gelatinous metastasis in the choroid of a cancer of the rectum was observed in a woman of 43 years 10 years after radical removal of the primary growth. As it was impossible to determine the metastatic nature of the ocular growth diagnosed ophthalmoscopically, enucleation was performed. Metastatic lesions in the skull and liver developed 2 years later.—M. J. E.

Ear

GARDNER, W. J., and O. TURNER. [Cleveland Clinic, Cleveland, O.] BILATERAL ACOUSTIC NEUROFIBROMAS. FURTHER CLINICAL AND PATHOLOGIC DATA ON HEREDITARY DEAFNESS AND RECKLINGHAUSEN'S DISEASE. Arch. Neurol. & Psychiat., 44:76-99. 1940.

Observations are given on 4 members of a family in which bilateral neurofibromas of the acoustic nerve are inherited as a dominant mendelian trait. The disease affects both male and female individuals, and the outstanding symptom is long-standing deafness. Some members are known to be deaf, but do not appear otherwise inconvenienced. Tumors of the eighth nerve in 2 individuals were associated with neurofibroma in other areas of the central nervous system. In the present series the diagnosis was verified at necropsy in 2 patients, while in 2, with clinical evidence of bilateral growths, a tumor of one side was removed surgically because of severe symptoms. One of the latter group belonged to the sixth generation of this family. Photographs of gross specimens and photomicrographs are reproduced.—M. J. E.

RICHTER, H. [Hals-Nieren-Ohren-Klin., Univ. Erlangen, Erlangen, Germany] ÜBER OHRGESCHWÜLSTE UND AUSWIRKUNG OHRFERNER TUMOREN AUF DIE FUNKTION DES GEHÖRORGANES. [AUDITORY TUMORS AND THE EFFECTS OF DISTANT TUMORS ON THE AUDITORY FUNCTION.] Monatschr. f. Krebsbekämpf., 9:25-32. 1941.

The paper contains a general discussion of primary and metastatic tumors of the ear and the effects of intracranial neoplasms on auditory function.—M. J. E.

ROSENWASSER, H. [Mt. Sinai Hosp., New York, N. Y.] NEOPLASMS INVOLVING THE MIDDLE EAR. Arch. Otolaryng., 32:38-53. 1940.

Four cases are described. In each a tumor mass was visible in the auditory canal and involved the middle ear. Pain and paralysis of the facial nerve were the salient clinical features. The tumor in the first case, a squamous cell cancer, was resected. The patient died 1 month later and necropsy disclosed infiltration of the temporal lobe of the brain. The second patient had an adenocarcinoma, apparently primary in the ear, and died after 4 months despite intensive roentgen therapy. The third had a fibrosarcoma and remained tumor-free 4 years after roentgen irradiation, and the fourth a neurofibroma which was excised successfully. Photomicrographs are reproduced.—M. J. E.

Breast

CAMIEL, M. R., and H. BOLKER. [Brooklyn Cancer Inst., Brooklyn, N. Y.] CARCINOMA ERYSIPELATODES. Surg., Gynec. & Obst., 72:635-641. 1941.

Two typical cases of carcinoma erysipelatodes, which developed after mastectomy and were mistaken for postoperative inflammatory conditions, are reported, and the medicolegal importance emphasized. The metastatic process is a permeation of the subepidermal lymphatics and tissue spaces rather than of venous channels.—H. G. W.

CHRISTIE, A. C. [Georgetown Univ. Med. Sch., Washington, D. C.] THE DIAGNOSIS AND MANAGEMENT OF CANCER OF THE BREAST. Texas State J. Med., 36:722-728. 1941.

General clinical discussion.—M. J. E.

CHUMLEY, C. L. [Knoxville, Tenn.] TUMORS OF THE BREAST. J. Tennesee M. A., 34:210-216. 1941.

This is a general consideration of the clinical aspects of the problem.—M. J. E.

DAVISON, T. C., and F. F. RUDDER. [Emory Univ. Sch. of Med., Atlanta, Ga.] BREAST TUMORS. South. Surgeon, 9:697-709. 1940.

The authors follow the generally accepted methods of treatment of mammary tumors.—M. J. E.

FLEISCHER, A. J., and J. I. KUSHNER. [Bronx Hosp., New York, N. Y.] INHIBITION OF LACTATION. PERCUTANEOUS USE OF TESTOSTERONE. J. Clin. Endocrinol., 1:407-408, 1941.

Testosterone propionate is said to inhibit lactation but no data are included showing measurements of actual decrease in milk production.—J. B. H.

FOOTE, F. W., JR., and F. W. STEWART. [Memorial Hosp., New York, N. Y.] LOBULAR CARCINOMA IN SITU. A RARE FORM OF MAMMARY CANCER. Am. J. Path., 17: 491-495. 1941.

The authors have observed carcinomatous changes localized in the small lobular ducts in 2 instances among 300 specimens of carcinoma of the breast; in 12 other cases lobular carcinoma *in situ* was recognizable even though there had been infiltration throughout other parts of the breast. In the noninfiltrative cases there was no retraction of the nipple or fixation of the skin or of the nodule. Microscopically one finds a lobule or group of lobules in which the cells are large, somewhat loosely arranged, and are displaced towards the centers of the lumina in a disorderly fashion. Mitoses are rare and the cells are uniform and lacking in hyperchromatism. As this lesion occurs in multiple lobules simple mastectomy is essential; further procedures must depend on the finding of infiltration.—H. B.

GRAY, H. K., and G. A. WOOD. [Mayo Clinic, Rochester, Minn.] SIGNIFICANCE OF MAMMARY DISCHARGE IN CASES OF PAPILLOMA OF THE BREAST. Arch. Surg., 42: 203-208. 1941.

After reviewing 227 cases of papilloma of the breast, the authors conclude that any patient with discharge from the nipple should be treated surgically, because in 60% of all patients with malignant papillomas who had discharge from the nipple, no tumor was palpable.—G. De B.

GREENE, R. R., and J. I. BREWER. [Northwestern Univ. Med. Sch., Chicago, Ill.] RELATION OF SEX HORMONES TO TUMORS OF THE FEMALE REPRODUCTIVE SYSTEM. Am. J. Roentgenol., 45:426-445. 1941.

This is a documented review of the results of experimental work on animals and the clinical inference drawn from these results. A total of 164 articles is cited dealing with carcinoma of the breast, carcinoma of the cervix, carcinoma of the endometrium, carcinoma of the vulva, and with nonmalignant tumors of these structures.

Production of breast carcinoma in mice requires estrogen dosages equivalent to 50 to 500 times the physiological amounts, and the treatment must be extended over a period representing one-tenth to one-half of the total life span of the animal. Omitting questions of the known differences in the reactions of different species, if the various carcinogenic actions of the estrogens are assumed to apply equally to the human, it would be necessary to give a human female 120.0 mgm. of estradiol benzoate a week for a period of 10 to 30 years to obtain carcinoma of the breast as it is produced in the mouse.

On the basis of this survey the authors present the following general remarks:

"The cause of certain tumors in certain animals has been definitely established. The administration of certain dosages of estrogens for certain periods of time have caused specific tumors in the mouse, rat, guinea pig, and rabbit. Filterable viruses cause specific tumors in the fowl and in the rabbit. Certain chemical carcinogens cause specific tumors in the mouse, rat, rabbit, and dog.

"On the other hand, experimental evidence is difficult to obtain in the human. While certain tumors in the human may be due to specific chemical carcinogens (bladder tumors in dye workers), the general cause of tumors in the human is not known. Genetic factors, irritation, chemical carcinogens, filterable viruses, sex hormones, and even other etiological factors have all had their proponents. The various 'proofs' of the importance of each of these factors have of necessity been by inference only.

"The authors of this review do not believe that there is sufficient evidence to clearly establish that any one factor, such as estrogens, is the sole important etiological agent in the production of malignant or nonmalignant tumors in the human female. There is insufficient evidence available to lead to the assumption that estrogens, as produced in the body or as administered by the physician, have directly caused tumor formation in the human."—E. A. L.

HALLEY, E. P., and P. J. MELNICK. [St. Joseph's Hosp., Stockton, Calif.] PRE-OPERATIVE IRRADIATION IN CARCINOMA OF THE BREAST. Radiology, 35:430-438. 1940.

Twenty-one cases of carcinoma of the breast were irradiated over periods of from 11 to 49 days with fractional methods. The tumor doses were from 1,200 r to 4,500 r. From 1 to 45 days after the end of radiation, amputation of the breast was done and the tumors studied histologically for changes due to irradiation.

The early effect of irradiation was production of necrosis of the radiosensitive tumor cells which was most prominent within the first 2 weeks. Later, bizarre cell forms with irregular nuclei progressing to giant cells with calcifying nuclei were seen. Four to 5 weeks after the end of irradiation resumption of tumor growth occurred, although late irradiation effects, such as clumps of debris containing calcified giant cell nuclei, and foreign body giant cells, still persisted. Fibrosis of the tumor bed and blood and lymph vessel obliteration did not occur. The similarity between these observations and those previously demonstrated in experimental animals by the junior author is noted. It is imperative to operate early before the tumor resumes its growth.—E. A. L.

LIVINGSTON, S. K. [Hines, Ill.] ADENOCARCINOMA, SCIRRHOUS TYPE, OF THE LEFT MALE BREAST WITH GENERALIZED BONE METASTASIS. Am. J. Roentgenol., 45:589-590. 1941.

Case report.—E. A. L.

MATTHEWS, A. A. [Spokane, Wash.] CARCINOMA OF THE BREAST. West. J. Surg., 48:502-507. 1940.

A general discussion on diagnosis and treatment.—M. J. E.

SAPHIR, O. [Michael Reese Hosp., Chicago, Ill.] MUCINOUS CARCINOMA OF THE BREAST. Surg., Gynec. & Obst., 72:908-914. 1941.

From a study of the literature and of 9 cases of his own, the author concludes that mucinous carcinoma of the breast is not a single entity, but can be classed into at least 4 definite types of tumors. 1. The true mucinous carcinoma, with but few tumor cells remaining, is relatively benign. 2. Duct carcinoma with mucinous features, is the most common type. 3. Signet ring cell mucinous carcinoma is highly malignant. 4. Intracystic papilloma with mucinous features is relatively rare and nonmalignant.—H. G. W.

SCHENCK, S. G. [Brooklyn Cancer Inst., Brooklyn, N. Y.] THE MANAGEMENT OF CANCER OF THE BREAST WITH PRE-OPERATIVE AND POST-OPERATIVE IRRADIATION. Radiology, 36:315-323. 1941.

All cases suspected of cancer of the breast are subjected to a biopsy examination and started on radiation therapy immediately. A 200 kv. instrument is used with a 50 cm. distance and filtration of 2 mm. of copper and 1 mm. of aluminum. First, the affected breast is cross-fired through two ports directed tangentially to the chest wall. Two hundred r (measured in air) are given to each port daily until 2,000 to 2,600 r are delivered to each. The axilla is then treated through one or two ports until 1,200 to 1,400 r are delivered. The supraclavicular fossa is then similarly irradiated. The total dose here is from 1,600 to 2,000 r to each port. Six to 8 weeks later a radical mastectomy is performed on patients with disease limited to the breast or to the breast and axilla. The postoperative course is started 4 to 6 weeks after the surgery and is similar to the preoperative except that the dosage is kept within the lower limits. Irradiation sterilization is recommended for all menstruating patients. The method has been in use $2\frac{1}{2}$ years.—E. A. L.

SCHOREGGE, C. W. [Quain and Ramstad Clinic, Bismarck, N. Dak.] SIMPLE VERSUS RADICAL MASTECTOMY IN CARCINOMA OF THE BREAST. Journal-Lancet, 51:203-208. 1941.

Early diagnosis of cancer of the breast is exceptional. Even in the ideal situation when the patient applies for treatment promptly after being cognizant of a lump, the neoplasm has been present undoubtedly for an unknown length of time. Despite the satisfactory percentage of cures reported frequently from other institutions following radical mastectomy, the results obtained by the author have not been encouraging. Of an earlier group of 58 patients so treated 58% survived 3 years, 38% 5 years, and 17% 10 years postoperatively without evidence of tumor. In a second group of 73 cases, 39 patients were submitted to a similar operative procedure, while 34 had a simple mastectomy performed. Two courses of roentgen therapy of 1,900 r each directed through multiple portals in the involved area were administered postoperatively. The results in these 2 small groups were almost identical, as 12 patients died of metastases in the former and 11 in the latter. The postoperative interval varied from under 1 year to 10 years. Photographs of the resulting local condition in cured patients are reproduced.—M. J. E.

SENTURIA, H. R. [Memorial Hosp., New York, N. Y.] RELATIONSHIP OF ARTERIAL HYPERTENSION TO SURGICAL RISK IN BREAST CANCER. ANALYSIS OF 446 CONSECUTIVE RADICAL MASTECTOMIES. J. Missouri M. A., 38:22-24. 1941.

Of 446 patients on whom radical mastectomy was performed for cancer of the breast 95 had hypertensive cardiovascular disease. Hypertension did not influence adversely the operative mortality,—7 (1.57%) died postoperatively in the series as a whole and 1 (1.05%) in the hypertensive group.—M. J. E.

THOMASON, T. H. [Beall Clinic, Fort Worth, Tex.] THE TREATMENT OF CANCER OF THE BREAST. South. Surgeon, 9:900-906. 1940.

General remarks.—M. J. E.

WEISS, K. [Rönt.-Rad. Abt. des Städt. Krankenhaus, Karlsruhe, Germany] BEI REIHENUNTERSUCHUNGEN VON MÄNNERN GEFUNDENE MAMMATUMOREN. [BREAST TUMORS IN MEN FOUND IN GROUP EXAMINATIONS.] Monatschr. f. Krebsbekämpf., 9:12-13. 1941.

Medical examination of 440 men called for auxiliary military service disclosed a carcinoma of the breast in 1 patient, aged 50 years, and a fibroadenoma in a second, aged 30. The first patient was tumor-free 5 years after radical excision of the growth.—M. J. E.

WHITMORE, E. R. [George Washington Univ. Med. Sch., Washington, D. C.] FIBRO-EPITHELIAL TUMORS, CHRONIC CYSTIC MASTITIS, AND CARCINOMA OF THE BREAST. Virginia M. Monthly, 68:251-267. 1941.

A discussion of many of the controversial aspects of the pathology, relationships, clinical appearances, and treatment of benign and malignant lesions of the breast.—M.J.E.

Urinary System—Male and Female

BUGBEE, H. G. [New York, N. Y.] EXPLORATION OF CERTAIN RENAL TUMORS. J. Urol., 46:1-16. 1941.

The treatment of all renal tumors is nephrectomy, if possible, but inoperable renal tumors are often rendered operable by a preliminary course of deep x-ray therapy. Painless hematuria is probably the most important early symptom of renal tumor.—H. G. W.

COLBY, F. H. [Collis P. Huntington Memorial Hosp., Boston, Mass.] EVALUATION OF THE SUPERVOLTAGE TREATMENT OF BLADDER TUMORS. J. Urol., 45:337-341. 1941.

Bladder tumors treated by external radiation with one million volts, constant potential, have responded better than similar growths treated with lower voltage units. Well marked regression has occurred in about one-half the cases so treated with at least temporary disappearance of the tumor in about one-third. Symptoms such as bleeding and painful urination were relieved in about half the cases. In its present experimental stage it is doubtful if this agent is curative and it should not be regarded as a substitute for surgery.—H. G. W.

GASPÁR, I. A. [Rochester, N. Y.] MALIGNANT KIDNEY TUMORS. A STUDY OF CASES AT THE ROCHESTER GENERAL HOSPITAL DURING A TWELVE-YEAR PERIOD. New York State J. Med., 40:1209-1217. 1940.

In the above period, 13 cases of malignant renal tumors were observed. In only a single instance, however, was the histologic structure typical of hypernephroma. Adenocarcinoma and papillary or alveolar cancer were found in 11 patients, and 1 tumor was an embryonal adenomyosar-

coma. Only 3 patients of the 11 with operable growths treated by nephrectomy survived after 5 to 7 years.—M. J. E.

HIGGINS, C. C., and F. H. SHIVELY, JR. [Cleveland, Ohio] MALIGNANT RENAL NEOPLASMS IN CHILDREN. Arch. Surg., 42:386-394. 1941.

After reviewing the 26 cases of Wilms' tumor in this series, the authors conclude that preoperative irradiation followed by nephrectomy and postoperative irradiation is the best method of treatment.—G. DeB.

McDONALD, J. R., A. K. DOSS, and G. J. THOMPSON. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE URINARY BLADDER IMITATING SARCOMA. J. Urol., 46: 38-51. 1941.

A group of 9 neoplasms is reported which were histologically suggestive of sarcoma in some portions, but it was possible to demonstrate the epithelial origin of all. The short clinical history and poor results of treatment in these cases attests to the extremely malignant nature of all lesions of this type in the urinary bladder.—H. G. W.

PEARSE, R., and R. A. McCOMB. [Toronto Gen. Hosp., Toronto, Canada] THE TREATMENT OF INFILTRATING TUMOURS OF THE BLADDER. Canad. M. A. J., 43:106-110. 1940.

Infiltrating vesical tumors were treated by partial cystectomy in 36 cases, cystotomy and diathermy with or without radon implantation in 68 cases, cystectomy and ureterosigmoidostomy in 18. The operative mortality was high in each instance and there was a considerable percentage of deaths within 1 year. A number of patients in each group survived for varying intervals. Of the 26 in the group treated by diathermy and suprapubic radon insertion 9 were free from disease after 2 to 4 years.—M. J. E.

PILCHER, F., and D. S. MACNAB. [Calgary, Canada] PRIMARY CARCINOMA OF THE URETER. Canad. M. A. J., 44:361-363. 1941.

A primary carcinoma of the ureter produced pain and hematuria. An attempt to catheterize the affected ureter disclosed an obstruction, and a filling defect appeared in a ureterogram. The involved ureter and corresponding kidney were removed in 2 stages. No evidence of a recurrence had arisen after 2 years.—M. J. E.

PRIESTLEY, J. T. [Mayo Clinic, Rochester, Minn.] GENERAL CONSIDERATIONS IN THE SURGICAL TREATMENT OF CARCINOMA OF THE BLADDER WITH PARTICULAR REFERENCE TO TOTAL CYSTECTOMY. New York State J. Med., 40:1441-1445. 1940.

Of 25 patients subjected to cystectomy (generally following preliminary ureterosigmoidostomy) for bladder cancer, 7 died postoperatively.—M. J. E.

SENGER, F. L., and J. J. BOTTONE. [Long Island Coll. of Med., Brooklyn, N. Y.] BENIGN PAPILLOMA OF THE RENAL PELVIS. Am. J. Surg., 53:125-128. 1941.

Case report.—H. G. W.

SMITH, E., and A. YOUNG. [Royal Victoria Hosp., Montreal, Canada] KIDNEY TUMOURS (AN ANALYSIS OF A SERIES OF 118 CASES). Canad. M. A. J., 44:149-152. 1941.

Benign tumors were encountered in 21 patients, the diagnosis being established postoperatively. Cysts constituted the common lesion in this group; more unusual were fibroma, papilloma, hemangioma, or leiomyoma. Removal invariably produces a complete cure. Operation was performed on 62 of 97 patients with malignant growths. Histologically these were classified as adenocarcinoma in 40 cases, carcinoma in 15, sarcoma in 3, embryoma (Wilms' tumor) in 2, and papillary carcinoma in 2. The diagnosis unfortunately is generally delayed, and the tumors are prone to metastasize. The ultimate prognosis is poor. All patients died of recurrences or metastases, the majority within 5 years, a few at a somewhat later date.—M. J. E.

Oral Cavity and Upper Respiratory Tract

CLERF, L. H. [Philadelphia, Penn.] CANCER OF THE LARYNX: AN ANALYSIS OF TWO HUNDRED AND FIFTY OPERATIVE CASES. Arch. Otolaryng., 32:484-498. 1940.

Two types of operative procedure were employed in cases of laryngeal cancer,—laryngofissure and laryngectomy. The former is the method of choice when the tumor is intrinsic and limited to the vocal cord. Of 149 cases in this group 7 patients died postoperatively and 20 had recurrences or metastases 1 to 12 years after operation. An estimate of the 3-year survival period was possible in 104 patients and 62 were free from disease at this time. Laryngectomy was employed in 101 cases for more extensive cancers. Six patients died postoperatively, and recurrence or metastases occurred in 20. Of 55 patients in this group submitted to operation 3 or more years previously, 26 survived without evidence of tumor. Photographs of excised gross specimens are reproduced.—M. J. E.

CHRISTIE, A. C. [Georgetown Univ. Med. Sch., Washington, D. C.] THE DIAGNOSIS AND TREATMENT OF CANCER OF THE PHARYNX. Tex. State J. Med., 36:490-494. 1940.

Diagnostic methods and technic of roentgen therapy are outlined.—M. J. E.

EHRLICH, D. E., and S. D. BLUM. [New York City Cancer Inst., New York, N. Y.] CARCINOMA OF THE TONGUE WITH METASTASES TO THE LUNGS. Rev. Gastroenterol., 8:211-218. 1941.

Squamous cell carcinoma of the tongue with lung metastasis is rare, occurring but twice in a series of 143 cases which are here reported.—H. G. W.

FAIER, S. Z. [Omaha, Neb.] HIGHLY MALIGNANT TUMORS OF THE NASOPHARYNX. Nebraska M. J., 25: 297-298. 1940.

One case each of transitional cell cancer and lymphoepithelioma is recorded. Roentgen therapy in each instance produced striking improvement, but the first patient died of an intracranial metastasis, while the second had a local recurrence.—M. J. E.

FOSTER, J. H. [Houston, Tex.] SELECTIVE TREATMENT OF CANCER OF THE LARYNX. Tex. State J. Med., 36:370-372. 1940.

A discussion of the surgical and roentgen methods of treatment of laryngeal cancer.—M. J. E.

FRIEDELL, H. L., and L. M. ROSENTHAL. [Chicago Tumor Inst., Chicago, Ill.] THE ETIOLOGIC ROLE OF CHEWING TOBACCO IN CANCER OF THE MOUTH. J. A. M. A., 116:2130-2135. 1941.

In support of the role of tobacco as an etiologic factor in cancer is the fact that cancer of the mouth in chewers develops at the point at which the chew is held, as was true in the eight cases reported. Perhaps tobacco is responsible for betel nut chewer's cancer, for tobacco is a common

ingredient of the betel nut chew. The chewer's cancer is preceded by leukoplakic changes, and metastasizes late in the disease, so that radiation therapy is usually successful. —H. G. W.

GATEWOOD, E. T. [Richmond, Va.] DIAGNOSIS AND SELECTION OF TREATMENT FOR CARCINOMA OF THE LARYNX. Virginia M. Monthly, 68:330-335. 1941.

The indications for surgery and radiotherapy in patients with laryngeal cancer are discussed. Laryngofissure or laryngectomy are preferred for intrinsic tumors depending upon their size and penetration, and in selected instances of extrinsic cancer. Irradiation is reserved for intrinsic lesions invading muscle with fixation, the more malignant extrinsic types, and for tumors associated with metastases. Drawings of operative technics, and photographs of excised masses and patients are reproduced.—M. J. E.

HALPERT, B. [Louisiana State Univ. Sch. of Med., New Orleans, La.] LIPOMA OF THE TONGUE. Arch. Path., 31: 510-511. 1941.

A lipoma of the tongue in a 55-year-old Negro woman is reported, apparently the first such growth in a Negro.—H. G. W.

JACKSON, C., and C. L. JACKSON. [Philadelphia, Pa.] CANCER OF THE LARYNX: ITS INCREASING INCIDENCE. Arch. Otolaryng., 33:45-65. 1941.

Analysis of the more accurate mortality statistics compiled in recent years indicates a constant increase in the incidence of laryngeal cancer. In the United States during the 5-year period of 1934-38 the proportion of deaths from cancer of the larynx of the total deaths from malignant disease increased steadily from 1 in 122 to 1 in 111. Cancer of the larynx occurs preponderantly in males in the ratio of approximately 10:1. The suggested causes of the greater frequency of laryngeal cancer are abuse of alcoholic beverages and tobacco, and increased contact of the general population with gasoline fumes and the dust of tarred roads. The report includes appropriate tables and graphs.—M. J. E.

JANES, R. M. [Toronto Gen. Hosp., Toronto, Canada] THE TREATMENT OF TUMOURS OF THE SALIVARY GLANDS BY RADICAL EXCISION. Canad. M. A. J., 43: 554-559. 1940.

Mixed tumors of the salivary glands are usually relatively benign growths. While occurring most commonly in the parotid, they are observed occasionally in the submaxillary gland, palate, or inner side of the cheek. Adequate surgical excision affects a high percentage of cures, but a recurrence is not infrequent following simple enucleation. The tumors are extremely radioresistant. The author's technic for the treatment of mixed tumors consists in a more radical excision which includes the surrounding portion of the normal parotid gland. A preliminary dissection of the facial nerve is made to insure thorough exposure of the tumor. Permanent injury to the nerve may generally be avoided. When the tumor is malignant, complete extirpation of the gland is indicated, and if necessary, branches of the facial nerve are sacrificed. Of the 9 patients with malignant forms, 1 was without evidence of a recurrence 1½ years following operation of the latter type. Photographs of patients and an excised tumor are reproduced.—M. J. E.

LAHEY, F. H., and H. F. NELSON. [Lahey Clinic, Boston, Mass.] BRANCHIAL CYSTS AND SINUSES. Ann. Surg., 113:508-512. 1941.

Twenty-seven cases of branchial cysts and sinuses were treated by surgical excision at the Lahey Clinic in the past 10 years. There were no recurrences in the group, despite the fact that one-third of the cases had unsuccessful treatment elsewhere. The embryology and differential diagnosis of these lesions is discussed. The operative technic is pictured. There is a bibliography of 8 papers.—A. M.

LOOPER, E. A. [Baltimore, Md.] THE DIAGNOSIS AND SURGICAL TREATMENT OF CARCINOMA OF THE LARYNX. South. Surgeon, 9:513-521. 1940.

General remarks on diagnosis and surgical technic.—M. J. E.

MARTIN, H., and E. L. SUGARBAKER. [Memorial Hosp., New York, N. Y.] CANCER OF THE TONSIL. Am. J. Surg., 52:158-196. 1941.

A special article based on a clinical study of 157 cases admitted to Memorial Hospital from 1931 to 1935. The etiology, clinical course, method of treatment, end results and prognosis are discussed in detail. The net 5-year cure rate is 18%, but in cases without metastasis it is 40%, while in those with metastasis it is 8%. Among the cases are 20 of lymphosarcoma, with 5-year cures in 20%. No evidence that chronic tonsillitis, smoking, or syphilis play a role in the etiology could be found, except that isolated cases of cancer of the base of the tongue, tonsil, and soft palate occur with sufficient frequency in heavy cigar smokers to support the theory of a direct causal relation.—H. G. W.

MATTICK, W. L. [State Inst. for the Study of Malignant Diseases, Buffalo, N. Y.] THE DIAGNOSIS AND TREATMENT OF CANCER OF THE TONSIL. Radiology, 35:268-273. 1940.

This is a report of 162 cases of carcinoma of the tonsil seen from 1915 to 1935. Methods of clinical and pathological diagnosis are reviewed. In the common squamous cell type of tumor the tonsil is indurated, enlarged, ulcerated, and fungating. In a small number of cases the tonsil may be hypertrophied with little or no ulceration, and when associated with early metastatic involvement of the upper anterior cervical triangle lymph nodes, suggests the diagnosis of anaplastic, transitional, or lymphoepithelioma type of disease.

Treatment should be irradiation. Generally this can be done with 200 kv. x-ray therapy. The tonsil and gland-bearing areas should be cross-fired with about 3,500 r, measured in air, delivered to each port. After subsidence of the mucositis, the residual primary tumor should be implanted with gold radon seeds or small radium needles. After the primary has healed, pathologically demonstrable disease in the cervical lymph nodes may be treated by removal of the nodes and implantation of gold radon seeds in the tissue bed if the capsule of the nodes is not involved. If the capsule is involved, it is best to seed the nodes *in situ*.

In the group without demonstrable cervical metastases the results have improved from 14% 3-year arrests in the patients treated from 1915-29 to 44% in those treated from 1932-35. In the group with cervical gland involvement the results have improved from 1.4% to 19.3% 3-year arrests.—E. A. L.

NEW, G. B., and H. E. DORTON. [Mayo Clinic, Rochester, Minn.] SUSPENSION LARYNGOSCOPY IN THE TREATMENT OF MALIGNANT DISEASE OF THE HYPOPHARYNX AND LARYNX. Surg. Gynec. & Obst., 72:930-935. 1941.

A selected group of 38 cases, in contrast to about 450 cases treated by operation, have been treated at the Mayo Clinic with the aid of suspension laryngoscopy. Of the 27 patients with malignant disease of the hypopharynx, 51.8% are still well, and of 11 patients with interlaryngeal lesions 90% are well.—H. G. W.

NEW, G. B., and J. B. ERICH. [Mayo Clinic, Rochester, Minn.] TUMORS OF THE NOSE AND THROAT. Arch. Otolaryng., 32:123-158. 1940.

This is a survey of the literature for the years 1938 and 1939.—M. J. E.

SINGLETON, A. O., and N. DUREN. [Univ. of Texas, Galveston, Tex.] TUMORS OF THE SALIVARY GLANDS. Texas State J. Med., 36:784-792. 1941.

The authors discuss tumors of the salivary glands and related tissues on the basis of 62 personally observed cases. Seven tumors were metastatic. Of the 55 primary growths 40 were mixed tumors and 15 carcinomas. Since approximately 33% of mixed tumors treated by simple excision recur, as contrasted with a rate of recurrence of only 5 to 10% following a radical resection, the latter procedure is advocated. Permanent total or partial facial palsy may be a complication of extensive surgery. Four patients were alive and tumor-free 4 months to 1 year following radical resection of malignant tumors.—M. J. E.

WALKER, G. W., and M. F. STOCK. [Fresno, Calif.] FIBROMA OF THE NASOPHARYNX. California and West. Med., 54:10-12. 1941.

Nasopharyngeal fibroma, while histologically a benign lesion, is clinically malignant or potentially so because of the enormous size it may attain with consequent obstruction of the nasal passage, its propensity to penetrate the bones of the base of the skull, and the tendency to profuse bleeding when subjected to surgical measures. By preliminary irradiation some reduction in size of the tumors may be achieved and the possibility of hemorrhage minimized, thus making a surgical intervention less hazardous. Three cases are reported illustrating this combined treatment. Radium (800-1,000 mgm. hr.) was introduced in a postnasal pack about the tumors, and 3 to 4 weeks later the subsisting portions were excised with comparative ease.—M. J. E.

WOODWARD, F. D., and V. W. ARCHER. [Univ. of Virginia, Dept. of Med., Charlottesville, Va.] THE RESULTS OF TREATMENT OF MALIGNANT TUMORS OF THE LARYNX, HYPOPHARYNX, NASOPHARYNX AND SINUSES. Virginia M. Monthly, 67:751-755. 1940.

The results of surgical and roentgen therapy in 50 cases of cancer of the larynx, pharynx, and sinuses are tabulated. A small number of patients in each group treated in early stages survived for periods of 6 months to 5 years.—M. J. E.

Salivary Glands

ŠKORPIL, F. [Prague] ÜBER DAS SPEICHELDRÜSEN-ADENOM. [SALIVARY GLAND ADENOMA.] Virchows Arch. f. path. Anat., 306:714-736. 1940.

Four examples of uncomplicated adenoma of the parotid gland are recorded. The growths in each instance were encapsulated and readily excised. In 3 cases the tumor acini were formed of serous cells with which were associated occasional acidophilic forms. In the fourth case, however, the oxyphilic cell type with foamy cytoplasm predominated. The latter cell occurs normally in the salivary glands, especially in advanced age.—M. J. E.

Intrathoracic Tumors—Lungs—Pleura

BENEDICT, E. B., and B. CASTLEMAN. [Massachusetts General Hosp., Boston, Mass.] SARCOIDOSIS WITH BRONCHIAL INVOLVEMENT. REPORT OF A CASE WITH BRONCHOSCOPIC AND PATHOLOGICAL OBSERVATIONS. New England J. Med., 224:186-189. 1941.

The authors present this as the first case of intrabronchial sarcoid reported in which diagnosis was established by bronchoscopy and biopsy. The patient had generalized sarcoidosis; lymph node and skin biopsies were also diagnosed as sarcoid. Two microscopic photographs and a bronchoscopic view are shown and 5 references given.—A. M.

BISGARD, J. D. [Omaha, Nebr.] A CASE OF BRONCHOGENIC CARCINOMA SUCCESSFULLY TREATED BY TOTAL PNEUMONECTOMY. Nebraska M. J., 26:13-15. 1941.

A patient of 61 years appeared in good condition 8 months following total extirpation of the right lung. The upper lobe was completely infiltrated by a bronchogenic cancer.—M. J. E.

BRAHDY, L. [Division of Occupational Diseases and Injuries of Municipal Employees, New York, N. Y.] LEIOMYOMA OF THE LUNG. Am. Rev. Tuberc., 43:429-434. 1941.

Report of a case of leiomyoma of the lung, removed surgically, in the presence of active tuberculosis.—H. G. W.

BRAUND, R. R., and H. E. MARTIN. [Memorial Hosp., New York, N. Y.] DISTANT METASTASIS IN CANCER OF THE UPPER RESPIRATORY AND ALIMENTARY TRACTS. Surg. Gynec. & Obst., 73:63-71. 1941.

In 284 cases of cancer of the upper respiratory and alimentary tract coming to autopsy, there was found 23.3% of blood-borne dissemination, the most common sites being the lung, the liver, and the pleura in the order named. Patients who died from cancer of the cheek, gingiva, palate, nasal cavity, and esophagus showed the greatest incidence of distant metastasis. It was impossible to determine the probability of distant metastasis from the morphology of the tumor or the age of the patient, but it was $2\frac{1}{2}$ times as frequent in patients with cervical lymph node involvement at the time of admission.—H. G. W.

BRESLIN, L. J. [Toronto, Canada] A SULCUS TUMOUR. Canad. M. A. J., 44:56-57. 1941.

A case of superior pulmonary sulcus tumor is described with the characteristic symptomatology of pain in the shoulder radiating to the arm, atrophy of the small muscles of the hand, Horner's syndrome, and roentgen evidence of a pulmonary mass. The tumor was inoperable, and microscopic examination of a fragment disclosed an undifferentiated carcinoma.—M. J. E.

CABITT, H. L. [Beth Israel Hosp., Boston, Mass.] HEMOTHORAX: ITS RELATION TO PRIMARY CARCINOMA OF THE LUNG. J. THORACIC SURG., 10:590-599. 1941.

The incidence of hemothorax in 247 proved cases of primary carcinoma of the lung was 35, whereas of 86 cases of hemothorax but 2 occurred in secondary carci-

noma of the lung. Surgical intervention is contraindicated in hemothorax with carcinoma cells or when malignancy is demonstrated by bronchoscopy in the presence of hemothorax.—H. G. W.

DIAMOND, S. [Veterans Administration Facility, Legion, Tex.] CLINICAL DIAGNOSIS OF PRIMARY CARCINOMA OF THE LUNG. Am. Rev. Tuberc., 43:713-722. 1941.

Clinical study.—H. G. W.

EVELETH, M. S., and N. C. WETZEL. [Western Reserve Univ., Cleveland, Ohio] RAPID GROWTH OF A BRONCHIOGENIC CARCINOMA. Cancer Research, 1:721-723. 1941.

Following pneumonectomy in a white male 39 years old, with no residual tumor observed in the thorax at the time of operation, a bronchiogenic carcinoma simplex attained a weight of 2500 gm. at the time of death 58 days later. Metastases were found in regional lymph nodes, pericardium and veins of esophagus, together with direct extension to the subcutaneous tissues beneath the surgical incision. Computation shows that the growth of the tumor must have been much more rapid in the later days than in the earlier days, probably attaining an ultimate value of about 340 gm. per day.—Authors' summary.

FARBEROV, B. E., and E. A. BASLOW. [Ukranian State Roentgen Radium Oncological Institute, Kharkov, U. S. S. R.] PRIMARY TUMORS OF THE LUNGS; ROENTGEN DIAGNOSIS AND THERAPY. Am. J. Roentgenol., 45:701-713. 1941.

Clinical report of 130 cases of primary carcinoma of the lung.—E. A. L.

GEBAUER, P. W. [Cleveland City Hosp., Cleveland, Ohio] THE DIFFERENTIATION OF BRONCHIOGENIC CARCINOMAS. J. Thoracic Surg., 10:373-395. 1941.

Based on a study of 216 cases the conclusion is drawn that pathologically and clinically small cell carcinoma, adenocarcinoma, and squamous cell carcinoma are 3 fundamental types of bronchiogenic cancer. In cases of small cell carcinoma, because of its central location, rapid growth, and potent invasive and metastatic powers, there is little hope that many cases will ever be cured by excision. Adenocarcinomas, which arise in the periphery of the lung, are most favorable for excision but are inaccessible for tissue examination. Squamous cell carcinoma is the best suited for surgical removal. The differences between these types early in the disease sometimes permit their distinction, and late in the disease approximately 60% differentiation is possible. Bronchoscopy will be negative in 40 to 50% of cases if performed at the outset of the symptoms. The impression that clinical symptoms tend to occur early when the tumor is in an operable state, has been gained from this study. This paper is followed by an extended discussion.—H. G. W.

GERUNDO, M. [State Hosp., Topeka, Kans.] TUMORS OF PLEURA AND PERITONEUM ANATOMO-HISTOLOGICAL STUDY. J. Kansas M. Soc., 42:18-26. 1941.

Three cases of malignant tumors derived from the lining cells of the pleura and 2 from the peritoneum are recorded. They are classified as mesothelioma.—M. J. E.

HAYTHORN, S. R., W. B. RAY, and R. A. WOLFF. [Allegheny Gen. Hosp., Pittsburgh, Penn.] PRIMARY FIBROMYXOSARCOMAS OF THE HEART AND PULMONARY ARTERY. Am. J. Path., 17:261-271. 1941.

A brief review is given of the cases in the literature of primary myxomas, fibromyxomas, and fibromyxosarcomas of the heart, with discussion of the theories of the origin of such tumors. The author's case is that of a 51-year-old woman whose symptoms of dyspnea and pain about the heart began 8 months before death. At postmortem examination there were found 3 myxomatous polyps, the largest of which measured 3 by 2.5 cm., which arose from the posterior wall of the pulmonary artery about 3 cm. above the valve cusps. Microscopically these were composed of large, spongy, round and spindle-shaped cells many of which were in mitosis; there were also neoplastic giant cells present. Extensive metastases were found along the pulmonary artery tree, within the parenchyma, and within the bronchi of both lungs; the microscopic structure of these was the same as that of the polyps in the main pulmonary artery.

Since there was an area of pulmonary arteritis around the base of the polyps the authors felt that the tumor might have arisen from a zone of myxomatous metaplasia in the organizing thrombus at this site, which subsequently underwent a sarcomatous change and metastasized to the lungs. (A leiomyoma of the uterus was also found but no unusual growth activity was present in it.) This is probably the second fibromyxosarcoma that has been described in the pulmonary artery, the authors state.—H. B.

KENWELL, H. N., and H. E. VOGEL. [Millard Fillmore Hosp., Buffalo, N. Y.] THYMOMA—AN UNUSUAL CASE. Am. J. Surg., 52:331-337. 1941.

A mediastinal tumor with endothelial characteristics is reported. It was removed, but with fatal outcome.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27052. New England J. Med., 224:207-210. 1941.

A case of dermoid cyst of the anterior mediastinum is discussed. The lesion, 20 cm. in diameter, was successfully removed.—A. M.

NEUBERGER, K. [Univ. of Colorado Sch. of Med., Denver, Colo.] PRIMARY MULTIPLE ALVEOLAR CELL TUMOR OF THE HUMAN LUNG. J. Thoracic Surg., 10:557-565. 1941.

A case of multiple primary "alveolar cell tumor" in the lung is described, and an attempt made to trace the origin of the tumor cells from the septal cells of the alveolar walls, similar to the common lung tumors of mice.—H. G. W.

OCHSNER, A., and M. DeBAKEY. [Tulane Univ., Sch. of Med., New Orleans, La.] CARCINOMA OF THE LUNG. Arch. Surg., 42:209-258. 1941.

The authors summarize for neoplastic disease of the lung the results of 116 pneumonectomies, including 15 of their own, which have been reported to date. In 109 of the collected cases in which a statement was made as to the outcome, 45 (41.3%) patients recovered and 64 (58.7%) died. Of the 45 patients who recovered from the operation, 8 have subsequently died. The authors believe the increased incidence of pulmonary carcinoma is real, and in support of this, they quote many autopsy series which in general show that the incidence of carcinoma of the lung in all autopsies and among all car-

cinomas has increased significantly. Although they admit that this increase is due partly to an increase in life expectancy and to better methods of diagnosis, they are convinced that the real cause is the increase in smoking, especially of cigarettes. The pathology, the symptomatology, the diagnosis, and the treatment of carcinoma of the lung are discussed. There are 10 graphs and a bibliography of 16 pages.—G. De B.

SAMSON, P. C., and E. HOLMAN. [Stanford Univ. Sch. of Med., San Francisco, Calif.] PRIMARY CARCINOMA OF LUNG: THE IMPORTANCE OF EARLY DIAGNOSIS IN INCREASING OPERABILITY AND CURABILITY. California & West. Med., 55:208-211. 1940.

After stressing the cardinal importance of early diagnosis of pulmonary cancer with the aid of adequate roentgen and bronchoscopic studies the authors report the results of pneumonectomy in 6 cases. Two patients died postoperatively of complicating purulent pericarditis and empyema respectively, 1 of cerebral metastases after 8 months, and 1 of coronary thrombosis after 1 year without evidence of tumor. Two are reported as symptom-free at an unstated time after operation.—M. J. E.

SCHATTENBERG, H. J., and J. F. RYAN. [Tulane Univ. Sch. of Med., New Orleans, La.] LYMPHANGITIC CARCINOMATOSIS OF THE LUNGS. Ann. Int. Med., 14:1710-1721. 1941.

Case report with extensive review of the literature. The signs and symptoms caused by the metastatic foci often overshadow the primary lesion. A significant incidence of Krukenberg tumor of the ovary has been noted, and sometimes blood dyscrasias from metastatic involvement of the bone marrow.—H. G. W.

Gastrointestinal Tract

ASHWORTH, C. T., and S. A. WALLACE. [Baylor Univ. Coll. of Med., Dallas, Tex.] UNUSUAL LOCATION OF CARCINOID TUMORS. Arch. Path., 32:272-276. 1941.

A review of the literature reveals reports of 25 carcinoid tumors in unusual locations; *i.e.,* in sites other than the appendix and small intestine. Three additional carcinoids are reported, one in Meckel's diverticulum, one in the colon, and one in the mesentery of the small intestine. Of the recorded carcinoids in unusual locations, 25% have metastasized. This is in close agreement with the number of carcinoids of the small intestine which metastasize, namely, 22.5%.—Authors' summary.

BERNSTEIN, A. [Michael Reese Hosp., Chicago, Ill.] CARCINOMA OF THE CARDIA. Rev. Gastroenterol., 8:234-238. 1941.

Case report.—H. G. W.

BEST, R. R. [Univ. of Nebraska Coll. of Med., Omaha, Nebr.] GASTRIC CARCINOMA: PREOPERATIVE, OPERATIVE AND POSTOPERATIVE MANAGEMENT. Nebraska M. J., 26:208-210. 1941.

Preoperative and postoperative medical care and technical procedures are discussed.—M. J. E.

COOPER, W. A. [Cornell Univ. Med. Coll., New York, N. Y.] THE PROBLEM OF GASTRIC CANCER. J. A. M. A., 116:2125-2129. 1941.

Analysis of the histories of 264 cases of cancer of the stomach shows that responsibility for the failure in treatment due to late diagnosis is divided among the patient (8 months), the patient's physician (4½ months), and the general hospital (1 to 6 months). The profession should abandon its fatalistic attitude towards this disease, due to delay in diagnosis.—H. G. W.

CORNELL, N. W., and L. A. HAUSER. [New York Hosp., New York, N. Y.] PRIMARY ADENOCARCINOMA OF THE JEJUNUM. Am. J. Surg., 53:177-180. 1941.

Report of a case successfully operated.—H. G. W.

GARLOCK, J. H. [Mt. Sinai Hosp., New York, N. Y.] THE PROBLEM OF CARCINOMA OF THE CARDIAC END OF THE STOMACH. Surg. Gynec. & Obst., 73:244-256. 1941.

The increasing incidence of successful resections for cancer of the esophagus has awakened interest in carcinoma of the cardiac end of the stomach. The author discusses the surgical problems, with reports of 15 operated cases, of which 5 were subjected to resection.—H. G. W.

GAULT, J. T., and P. KAPLAN. [Mt. Sinai Hosp., Chicago, Ill.] SUBMUCOUS LIPOMA OF THE COLON. Am. J. Surg., 53:145-151. 1941.

A case report of submucous lipoma of the transverse colon which simulated carcinoma.—H. G. W.

HAINES, C. [New Rochelle, N. Y.] PERFORATION OF CARCINOMA OF THE STOMACH. Rev. Gastroenterol., 8: 239-241. 1941.

Case report.—H. G. W.

HAYES, H. T., and H. B. BURR. [Houston, Tex.] CANCER OF THE RECTUM, SIGMOID AND ANUS. South. M. J., 34:806-812. 1941.

Clinical study of 40 cases.—H. G. W.

LAIRD, T. K. [Laird Memorial Hosp., Montgomery, W. Va.] CARCINOMA OF THE COLON IN A CHILD OF FOURTEEN YEARS. Am. J. Surg., 53:335-339. 1941.

Eighteen authentic cases of carcinoma are found reported occurring in children under the age of 15 years, and proximal to the rectosigmoid junction. To these is added a case of colloid carcinoma of the splenic flexure of the colon in a white male of 14, with fatal outcome.—H. G. W.

LOEWENBERG, S. A., and M. SEGAL. [Philadelphia, Pa.] PRIMARY CARCINOMA OF THE ILEUM WITH METASTASES TO THE GREAT OMENTUM. Rev. Gastroenterol., 8: 193-197. 1941.

Case report.—H. G. W.

McLAUGHLIN, C. W., JR., and W. M. DILWORTH. [Univ. of Nebraska Coll. of Med., Omaha, Nebr.] CARCINOMA OF THE RECTUM. Nebraska M. J., 26:16-21. 1941.

A radical resection was possible in only 25 of 60 patients with cancer of the rectum. Five were alive 3 to 28 months postoperatively.—M. J. E.

ORR, T. G. [Univ. of Kansas Hosp., Kansas City, Kans.] RESECTION OF DUODENUM AND HEAD OF PANCREAS FOR CARCINOMA OF THE AMPULLA. Surg. Gynec. & Obst., 73:240-243. 1941.

Report of the fifteen cases so far operated, with 4 survivors. The plan of the operation is fundamentally sound, but a study of many more patients operated on by the Whipple technic will be necessary before the value of the procedure can be determined.— H. G. W.

SCHINDLER, R., P. E. STEINER, W. M. SMITH, and M. E. DAILEY. [Univ. of Chicago, Chicago, Ill.] THE CLASSIFICATION OF GASTRIC CARCINOMA. Surg. Gynec. & Obst., 73:30-39. 1941.

Histological criteria, especially grading, seem not to have a direct correlation to the gross appearance of gastric carcinoma, to its clinical course, or to its surgical cura-

bility. Gross classification has proved satisfactory; namely, circumscribed polypoid growth, second, sharply limited noninfiltrating carcinomatous ulcer, and the third and fourth groups of infiltrative carcinomas. The preliminary impression is that gross types I and II often give excellent results after surgical interference, while such treatment is unfavorable for infiltrating types III and IV.—H. G. W.

SHARPE, W. S., and M. W. COMFORT. [Mayo Clinic, Rochester, Minn.] CARCINOMA OF THE PAPILLA OF VATER; CLINIAL FEATURES IN FORTY CASES. Am. J. M. Sc., 202:238-245. 1941.

This is a consideration of the diagnostic difficulties presented by carcinoma of the ampulla, a disease found in 0.2% of all autopsies. In this series 34 cases were in men and 6 in women. Increased awareness on the part of the surgeon of the frequency of the lesion may lead to eventual satisfactory surgical approach.—H. G. W.

VAUGHN, A. M. [Loyola Univ. Sch. of Med., Chicago, Ill.] PRIMARY CARCINOMA OF THE AMPULLA OF VATER. Am. J. Surg., 51:489-493. 1941.

Case report with review of the literature.—H. G. W.

VOLDENG, K. E. [Wellington, Kans.] PRINCIPLES NECESSARY IN THE SUCCESSFUL MANAGEMENT OF COLON CANCER. J. Kansas M. Soc., 42:97-102. 1941.

This is a general discussion designed for the general practitioner of the diagnosis and surgical treatment of cancer of the colon.—M. J. E.

WAKABAYASHI, O. [Imperial Univ., Tokyo] EIN FALL VON RETIKULOSARKOM IM RETROPERITONEUM 4 JAHRE NACH REKTUMKREBSOPERATION. [A CASE OF RETROPERITONEAL RETICULOSARCOMA 4 YEARS AFTER OPERATION FOR A RECTAL CANCER. Gann, 35:77-79. 1941.

A case is described of a patient who 4 years after surgical removal of a rectal carcinoma was found to have a large reticulosarcoma which invaded the stomach and pancreas, and had metastasized to the liver and retroperitoneal lymph nodes. This case is unusual in that 2 distinct types of malignant tumors were found in the same individual.—P. P. C.

WATSON, E. A., and D. P. WATSON. [Grand Island, Nebr.] FIBROMA OF TRANSVERSE COLON. REPORT OF ONE CASE. Nebraska M. J., 26:175-176. 1941.

A large fibroma, measuring 25 x 18 x 15 cm., was excised successfully from the region of the transverse colon of a man of 35 years. It was necessary to remove a portion of the attached bowel.—M. J. E.

Liver

GNASSI, A. M. [Med. Center, Jersey City, N. J.] PRIMARY LIVER CELL CARCINOMA. Am. J. Surg., 53:260-264. 1941.

In 2,870 surgical and post-mortem malignant tumors 4 cases of primary liver cell carcinoma were encountered, and herewith reported.—H. G. W.

GREENLEE, D. P., R. C. HAMILTON, and F. P. FERRARO. [Pittsburgh, Pa.] PRIMARY CARCINOMA OF THE GALL BLADDER. Arch. Surg., 42:598-610. 1941.

The authors present 5 cases of carcinoma of the gall bladder seen in a period of 1½ years. They recommend early operation for patients with benign disease of the gall bladder and with silent stones, because the risk is small. Only in this way will the incidence be reduced.—G. De B.

HANSEN, A. E., M. R. ZIEGLER, and I. McQUARRIE. [Univ. of Minnesota, Minneapolis, Minn.] DISTURBANCES OF OSSEOUS AND LIPID METABOLISM IN A CHILD WITH PRIMARY CARCINOMA OF LIVER. J. Pediat., 17:9-30. 1940.

A primary parenchymal cell carcinoma of the liver with metastases in the lungs and spleen is described in a boy of 10 years. The patient developed pronounced lipemia and lipid histiocytosis, as evidenced by the presence of large xanthoma-like cells, in the tumor and uninvolved areas of the liver, spleen, lungs, right kidney, and bone marrow. Extreme osteoporosis developed in all bones with subsequent deformities. The osseous changes resulted from defective retention by the body of calcium and phosphorus, possibly secondary to impaired hepatic function, as no hyperparathyroidism, vitamin D deficiency, or disturbed mineral absorption was detectable. Photographs of the patient and tumor, photomicrographs and charts are included in the report.—M. J. E.

VAN ZANDT, I. L. [Univ. of Texas, Galveston, Tex.] CARCINOMA OF THE GALL BLADDER. Texas State J. Med., 36:616-618. 1941.

In a series of 5,000 autopsies 546 examples of malignant disease were found, of which 9 were cancers of the gall bladder. With the exception of 1 squamous cell cancer all tumors of the gall bladder were adenocarcinomas. Associated cholelithiasis was found in 6 cases. Metastases were usually regional in distribution and present in each case.—M. J. E.

WENTZ, V. B., and K. KATO. [Univ. of Chicago, Chicago, Ill.] PRIMARY CARCINOMA OF LIVER WITH BANTI'S SYNDROME. J. Pediat., 17:155-165. 1940.

A case report of associated Banti's disease and primary cancer of the liver in a child of 6 years. The patient was known to have had an enlarged spleen since infancy. When 2½ years of age, a diagnosis of Banti's syndrome was made, but a splenectomy was delayed until the age of 6 years. The child died postoperatively. The liver was cirrhotic and contained multiple nodules of primary parenchymal cell carcinoma. A photograph of the patient and photomicrographs are included.—M. J. E.

Bone and Bone Marrow

BATTLE, J. D., JR., and J. STASNEY. [Sch. of Med., Louisiana State Univ., New Orleans, La.] MALIGNANT MELANOMA CELLS IN THE BONE MARROW. Arch. Path., 31:631-633. 1941.

A case is reported in which the finding of abnormal cells in the bone marrow on sternal puncture suggested diffuse metastases from malignant melanoma, verified by necropsy. —H. G. W.

BATTS, M., JR. [Univ. of Michigan Hosp., Ann Arbor, Mich.] PERIOSTEAL FIBROSARCOMA. Arch. Surg., 42: 566-576. 1941.

The author analyzes 27 cases of periosteal fibrosarcoma diagnosed at the above hospital since 1925. The mortality is about 50%, and the incidence of 5-year survivals is 40%, but all of the patients who survived 5 years or more had lesions of a low degree of malignancy. The clinical, roentgenological, and pathological features and the treatment are also discussed.—G. De B.

BOLDREY, E., and W. J. McNALLY. [McGill Univ. and Montreal Neurol. Inst., Montreal, Canada] CHORDOMA OF

THE BASSIOCCIPUT AND BASISPHENOID: REPORT OF FOUR CASES. Arch. Otolaryng., 33:391-400. 1941.

In the 4 cases of chordoma involving the base of the skull the tumor in 2 instances was confined to the interior of the cranial cavity and in 2 extended into the vault of the nasopharynx. The symptoms and signs were indicative of an intracranial neoplasm with evidence of multiple involvement of the cranial nerves. Roentgen signs of erosion of the base of the skull are generally present in cases of this type, especially when nasopharyngeal extension has occurred. A histologic diagnosis may be established by examination of a smear of material procured by aspiration through a large gauge hypodermic needle of the nasopharyngeal mass visualized directly with the aid of a nasopharyngoscope. The therapy is difficult, but the course in untreated or irradiated patients may be protracted as the tumor grows slowly. Operation was attempted in the 2 patients with a growth limited to the interior of the skull, but both died postoperatively. One of the patients in the second group was benefited by radium and roentgen therapy, and no treatment was attempted in the other because of the wide extension of the tumor.—M. J. E.

BOWERS, W. F. [Univ. of Nebraska Coll. of Med., Omaha, Neb.] SACRO-COCCYGEAL CHORDOMA—A CASE REPORT. Nebraska M. J., 25:341-342. 1940.

A resection of the sacrum was necessary in this case in order to remove a chordoma situated on the posterior surface of the rectum.—M. J. E.

CINELLI, A. A. [New York, N. Y.] OSTEOMA EBURNEUM OF THE MASTOID. Arch. Otolaryng., 33:421-424. 1941.

This is a case report of an osteoma attached to the mastoid cortex. The tumor was readily extirpated.—M. J. E.

COLEY, B. C. [New York, N. Y.] CONSERVATIVE SURGERY IN TUMORS OF BONE. South. Surgeon, 10:379-392. 1941.

Giant cell tumors, central chondroma, and localized sarcoma of bone of low grade malignancy may be treated conservatively by curettage of the tumor and introduction of transplants of bone fragments into the resulting cavity. Mutilating effects of more extensive surgical intervention are thus avoided. Roentgen therapy in many instances has also proved satisfactory. Eight cases are recorded in which conservative treatment was sufficient for a complete cure or temporarily arrested symptoms.—M. J. E.

CROCKETT, R. H. [San Antonio, Tex.] X-RAY TREATMENT OF EWING'S TUMOR. Tex. State J. Med., 36:417-422. 1940.

Temporary improvement for 1 year was achieved by roentgen therapy in a child of 5 years with a Ewing's tumor of the radius and pulmonary metastases.—M. J. E.

DeSANTO, D. A., R. TENNANT, and P. D. ROSAHN. [Hosp. for Ruptured and Crippled, New York, N. Y.] SYNOVIAL SARCOMAS IN JOINTS, BURSAE AND TENDON SHEATHS. Surg., Gynec. & Obs., 72:951-981. 1941.

A study of 16 cases of synovial sarcoma leads to the conclusion that all varieties exhibit specific characteristics which permit of their identification. An endothelial structure and function is frequently exhibited as gland-like spaces resembling adenosarcoma, as sheets or nests of epithelium-like cells, as neoplastic villi, or as well differentiated synovial borders. A histiocytic structure and function is exhibited by phagocytic properties, sometimes resembling xanthomatous and giant cell tumors. Synovial sarcoma may also appear indistinguishable from fibrosarcoma unless synovial clefts or small areas of reticuloendothelial evolution are located. Mucin formation is not infrequently exhibited. Clinically, synovial sarcomas in knee joints extend over a long insidious pre-operative course, but synovial sarcomas of other para-articular regions manifest themselves as soft part tumors after an average period of a few to several months. Amputation is the treatment of choice for all synovial sarcomas.—H. G. W.

EDWARDS, J. E. [Boston City Hosp., Boston, Mass.] PRIMARY RETICULUM CELL SARCOMA OF THE SPINE. Am. J. Path., 16:835-844. 1940.

The author presents the clinical and pathological findings of a patient with primary reticulum cell sarcoma of the spine, and summarizes many of the characteristics of this entity as described by Parker and Jackson (Surg., Gynec. & Obst., **68**:45-53. 1939) which this case showed. This patient was a 59-year-old woman who had suffered with back pain for a short time 3 years previously and steadily for 15 months before her death. She finally developed paralysis and loss of sensations in both legs. At autopsy a reticulum cell sarcoma was found involving the bodies of the 12th thoracic and 1st and 2nd lumbar vertebrae. It had invaded the lumbar epidural space and compressed the spinal cord. The para-aortic and iliac lymph nodes contained tumor as well. This tumor was designated as primary in the spine because of the duration of the symptomatology and the comparative bu'k of tumor in the spine and in the local lymph nodes.—H. B.

FIENBERG, R., and F. H. BAEHR. [Westfield State Sanitorium, Westfield, Mass.] HEMANGIOMA OF TIBIA WITH METASTASIS TO THE POPLITEAL ARTERY. Arch. Path., 31:811-818. 1941.

A primary tumor of the tibia is reported as an authentic malignant anaplastic hemangioma or hemangioendothelioma, which metastasized to the tibial artery. The diagnosis of this rare type of tumor of bone can be made only by biopsy, since the roentgenologic appearance simulates that of a benign giant cell tumor. It is suggested that not all tumors of the long bones diagnosed as hemangioma be regarded as benign, despite the opinion prevalent in the literature.—H. G. W.

FISH, E. W. [St. Mary's Hosp., Paddington, London, England] BENIGN NEOPLASIA OF TOOTH AND BONE. Proc. Roy. Soc. Med., 34:427-445. 1941.

This communication records a rare and apparently neoplastic lesion of the dental pulp and a series of cases showing a similarly destructive lesion originating in the parodontal membrane. In a summary of the nature of tumors of the dental tissues, the author points out that the only members of the group which are accepted as showing uncontrolled cell proliferation are the adamantinoma (epithelial tumor of the tooth bud) and the cementoma (parodontoma). The remaining odontomas have been regarded as developmental tumors arising in the course of aberrations of growth. However, two other morbid conditions are suggested as possibly representing neoplastic

change in the cells associated with the mature tooth, and these are described as endodontoma and odontoclastoma.

The paper possesses two features of more general value, *viz.*: 1. a comparison of two (suggested) symmetrical series, of bone tumors on the one hand (endosteoma, periosteoma, and osteoclastoma), and tooth tumors (endodontoma, parodontoma, and odontoclastoma) on the other; and 2. an interesting discussion of heterogeniture in relation to benign neoplasia in bone. Both topics are treated in considerable detail, and closely reasoned, so that they must be studied in the original.—A. H.

FOOTE, F. W., JR., and H. R. ANDERSON. [Memorial Hosp., New York, N. Y., and Memorial Hosp., Johnstown, Pa.] HISTOGENESIS OF EWING'S TUMOR. Am. J. Path., 17: 497-502. 1941.

One portion of a Ewing's endothelial myeloma arising in a rib consisted histologically of vascular spaces lined by elongated cells resembling endothelial cells. In other areas the structure most commonly seen in Ewing's tumor was present apparently resulting from diffuse overgrowth of these endothelial elements. In still other foci the same type of cell was arranged in rosettes. The histological structure noted first with the apparent development of the other histological pictures led the authors to conclude that Ewing's thesis of the origin of this tumor in vascular endothelium was sustained.—H. B.

GHARPURE, V. V. [Tata Memorial Hosp., Bombay, India] ENDOTHELIAL MYELOMA (EWING'S TUMOR OF BONE). Am. J. Path., 17:503-507. 1941.

The autopsy findings in a case of Ewing's tumor are presented. The tumor arose in the femur of a 14-year-old boy; there were two fractures at this site. One solitary metastasis was present on the external surface of the base of the right lung. Histologically the tumor consisted of large numbers of pseudo-rosettes between which there was a diffuse growth of polyhedral ceils; a perithelial arrangement was occasionally noted. There was no specific relation noted between the cells and reticulum fibers. The author feels that Ewing's tumor is an entity and that the histology of this case is consistent with Ewing's interpretation of its origin in capillary endothelium.—H. B.

HERZMARK, M. H. [Hosp. for Joint Diseases, New York, N. Y.] GIANT-CELL SYNOVIAL TUMOR OF THE KNEE. J. Bone & Joint Surg., 23:684-686. 1941.

Case report.—H. G. W.

HORWITZ, T. [Jefferson Med. Coll., Philadelphia, Penn.] CHORDAL ECTOPIA AND ITS POSSIBLE RELATION TO CHORDOMA. Arch. Path., 31:354-362. 1941.

The results of this investigation serve to corroborate and supplement the present knowledge of the variations in the regression of the human notochord, and the persistence of aberrant chordal tissue in the human fetus and embryo in the cranial, vertebral, and sacrococcygeal regions after birth. Such heterotopic chordal vestiges may be the starting point for neoplasia. Their topographic distribution corresponds closely to the sites of occurrence of chordoma and this is interpreted as supporting the view that chordoma arises from the aberrant chordal vestiges rather than from the chordal remnants within the nucleus pulposus of the intervertebral disk.—H. G. W.

JACOBSON, S. A. [The Richard Morton Koster Research Lab., Brooklyn, N. Y.] CRITIQUE ON THE INTER-RELATIONSHIPS OF THE OSTEOGENIC TUMORS. Am. J. Cancer, 40:375-402. 1940.

The author develops the thesis that "the uniform histogenesis of the supportive tissues would seem to presuppose a unity of their new growths, benign and malignant." The views of Geschickter and Copeland and others on the histogenesis of osteogenic bone tumors are discussed in the light of this concept.—L. L. W.

JOHNSON, G. S. [Nashville, Tenn.] THE TREATMENT OF CARCINOMA OF THE JAW. Surgery, 9:492. 1941.

About 50% of cases were reported well between 2½ and 5 years after operation for carcinoma of the jaw.—A. M.

KELLY, L. M. C. [New York, N. Y.] VERTEBRAL HEMANGIOMA WITH NEUROLOGIC SYMPTOMS. New York State J. Med., 40:1607-1612. 1940.

Pain in a patient with multiple vertebral hemangioma was alleviated by roentgen therapy.—M. J. E.

KINNEY, L. C. [San Diego, Calif.] MULTIPLE MYELOMA. Radiology, 35:667-675. 1940.

The typical case of multiple myeloma presents a fairly obvious picture, but there may be many variants. The most characteristic finding is the multiple bone lesions which most frequently involve the spine, ribs, skull, and pelvis. The lesions, however, may be absent in any one of these locations. They are small, clean-cut areas of bone destruction. Bence-Jones proteinuria occurs in from 50 to 65% of the cases but may also occur in any disease involving bone marrow including metastatic carcinoma. Nephritis occurs in 70% of the cases. The blood picture is not characteristic; there is usually progressive anemia; and there may be an increase in the serum calcium and a hyperproteinemia. Multiple myeloma must be differentiated from hyperparathyroidism and from the osteolytic type of metastatic carcinoma. A biopsy may frequently be the only means of making an early diagnosis.—E. A. L.

KLEINBERG, S. [Hosp. for Joint Diseases, New York, N. Y.] OSTEOID OSTEOMA OF THE FEMUR. Am. J. Surg., 53:168-171. 1941.

Case report.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORD OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27112. New England J. Med., 224:473-476. 1941.

A case of Ewing's tumor of the lower end of the fibula is presented and some features of the disease discussed.—A. M.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27131. New England J. Med., 224:559-563. 1941.

A case of multiple myeloma is reported and discussed. Bone x-rays were negative yet a high serum protein (10.7) and the finding of immature plasma cell elements on sternal puncture justified the clinical diagnosis. Autopsy showed diffuse plasma cell myeloma and a probable reticulum cell sarcoma of the femur.—A. M.

MYERDING, H. W., and J. E. VALLS. [Mayo Clinic, Rochester, Minn.] PRIMARY MALIGNANT TUMORS OF BONE. J. A. M. A., 117:237-243. 1941.

A study of 424 primary malignant tumors of bone encountered at the Mayo Clinic between 1909 and 1934. As to type they were distributed as follows: osteogenic sarcoma, 216; fibrosarcoma, 38; Ewing's sarcoma, 114;

multiple myeloma, 41; malignant giant cell sarcoma, 7; nonsurgical diagnosis of sarcoma, 8. The 5-year survivals were respectively: osteogenic sarcoma, 20.3%; fibrosarcoma, 30.5%; Ewing's sarcoma, 21.2%; multiple myeloma, 3.6%; malignant giant cell sarcoma, 83.3%. Males constituted 68.6%, and 58.8% were less than 30 years old. The femur was involved in 29.3%, the tibia in 14.2%, and the extremities were involved in 62.8%. Although the authors do not believe that trauma is an etiological factor, yet a positive history was given in 40.6%.—H. G. W.

NIELSEN, J. [Radiol. Abt. Reichsspitals, Copenhagen, Denmark] ZWEI FÄLLE VON EWING-SARKOM IM UNTERKIEFER. [TWO CASES OF EWING'S TUMOR OF THE LOWER JAW.] Acta Radiol., 21:286-291. 1940.

The unusual localization and success of therapy are of special interest in 2 cases of Ewing's tumor. In both patients a mass was present in the lower jaw producing roentgen evidence of bone destruction. It was possible to resect the involved half of the mandible of 1 patient, but a recurrence developed after 1½ years. Roentgen therapy was now administered. The tumor regressed completely, and the patient appeared symptom-free after 5 years. In this time an independent adenocarcinoma of the prostate was removed. The tumor in the second patient infiltrated the soft tissues of the cheek. As this was deemed inoperable, he received protracted fractionated roentgen irradiation (7,000 r total dose in 7 weeks through 3 portals). The mass gradually disappeared and no tumor was detectable clinically or roentgenographically 5 years later.—M. J. E.

PAUL, L. W., and E. A. POHLE. [Univ. of Wisconsin Med. Sch., Madison, Wis.] SOLITARY MYELOMA OF BONE. Radiology, 35:651-666. 1940.

Forty-one cases of solitary myeloma of bone reported in the literature are reviewed, and 4 new cases are added. Every case had a microscopic diagnosis from tissue removed either at biopsy or post-mortem examination. The dorsal spine (9 cases), pelvis (10 cases), and femur (8 cases) were the most frequent sites involved. The ratio of males to females was 3.5 to 1. The average age of the patients was 48. Bence-Jones protein was found in the urine of 9 patients. Two main types of the disease are distinguishable roentgenologically.

The disease may be relatively benign and remain localized or, it may undergo an early spread to form typical multiple myeloma. It is most commonly confused with simple giant cell tumor. The latter, however, is predominantly a lesion of the epiphyseal ends of bone.

Treatment has been surgical, radiological, or a combination of both. Twenty-eight patients were still living at the time of the report and 24 were free from evidence of spread.—E. A. L.

PIERSON, J. W., G. FARBER, and J. E. HOWARD. [Johns Hopkins Univ. Sch. of Med., Baltimore, Md.] MULTIPLE HEMANGIOMAS OF BONE, PROBABLY CONGENITAL. J. A. M. A., 116:2145-2148. 1941.

Report of a unique case of hemangioma involving many bones, apparently nonprogressive.—H. G. W.

RAWLINS, A. G. [San Francisco, Calif.] OSTEOMA OF THE MAXILLARY SINUS: REPORT OF A CASE. Arch. Otolaryng., 32:499-505. 1940.

The tumor in this case was successfully excised.—M. J. E.

SPENCER, F. R., C. P. HEGNER, and W. C. BLACK. [Univ. of Col. Sch. of Med. and Hosp., Denver, Col.] BENIGN AND MALIGNANT TUMORS OF THE JAW. Arch. Otolaryng., 32:200-245. 1940.

The authors record 19 cases. The lower jaw was involved in all but one patient. In the latter the tumor was situated in the upper jaw. Malignant tumors were present in 15 patients. These were advanced, inoperable growths frequently associated with distant metastases, with the exception of a single instance of a primary fibrosarcoma. This was extirpated radically and the patient was tumor-free 6 years later. Of the patients with inoperable growths, 2 had sarcomas apparently primary in the mandible, 1 a metastasis of a gastric cancer, and the remaining squamous cell cancers secondary to the lip or mucous membrane of the mouth. In some instances temporary alleviation was achieved by conservative surgical measures and radiotherapy. Of the group with benign neoplasia, 2 had epulis, and 1 patient was cured by excision of the mass and radium implantation. The second example of epulis occurred in a newborn infant and operation was deferred. A case each of dentigerous and sublingual cyst is included, and these lesions were cured promptly by a simple surgical intervention.—M. J. E.

STOKES, H. B. [Omaha, Neb.] PRIMARY MALIGNANT TUMORS OF THE TEMPORAL BONE: REPORT OF A CASE. Arch. Otolaryng., 32:1023-1030. 1940.

A case report of an extensive squamous cell cancer in the auditory canal which invaded the middle ear and mastoid process. The tumor was excised radically and postoperative radiotherapy instituted, but the patient died 5 months later of an intracranial metastasis.—M. J. E.

TEED, R. W. [St. Joseph's Mercy Hosp., Ann Arbor, Mich.] PRIMARY OSTEOMA OF THE FRONTAL SINUS. Arch. Otolaryng., 33:255-292. 1941.

Two cases are reported. The tumors were removed surgically. The paper contains a historical review of the subject, an extensive bibliography with photographs, and roentgenograms.—M. J. E.

TOLLMAN, J. P., and P. R. TEAL. [Univ. of Nebraska Coll. of Med., Omaha, Nebr.] MULTIPLE MYELOMA. Nebraska M. J., 26:141. 1941.

A report with autopsy record of a case of plasma cell myeloma involving the liver, spleen, and skeletal system.—M. J. E.

Muscle and Tendon

GRAYZEL, D. M., and H. H. FRIEDMAN. [Jewish Hosp., Brooklyn, N. Y.] MYOBLASTOMA OF THE THORACIC WALL. Arch. Path., 31:512-515. 1941.

A total of 78 cases of myoblastoma have been reported to date, to which is added another located in the thoracic wall, the first in this site.—H. G. W.

KYLE, B. H. [Lynchburg, Va.] OSSIFYING FIBROMA-THENAR SPACE. Virginia M. Monthly, 68:164. 1941.

A fibroma, in which secondary ossification was demonstrable roentgenographically, was excised with ease.—M. J. E.

Leukemia, Lymphosarcoma, Hodgkin's Disease

ABELS, J. C., J. M. KENNEY, L. CRAVER, L. D. MARINELLI, and C. P. RHOADS. [Memorial Hosp., New York, N. Y.] POSTIRRADIATION CHANGES IN THE LEVELS OF

ORGANIC PHOSPHORUS IN THE BLOOD OF PATIENTS WITH LEUKEMIA. Cancer Research, 1:771-775. 1941.

The administration of subtherapeutic amounts of radioactive phosphorus to 5 patients with leukemia has been followed by an alteration of the organic acid-soluble phosphorus fraction of their blood cells. The administration of nonradioactive phosphorus to 6 patients never was followed by any significant alteration of the organic acid-soluble phosphorus of the blood cells. These same alterations were observed after the administration of very small doses of whole body x-irradiation to 3 patients, and after irradiation of the blood through a precordial port to 3 patients. The amount of radiation delivered by the tracer doses of radioactive isotopes used in metabolism studies cannot be regarded as negligible. It is possible that some of these studies have measured the metabolism following radiation. —Authors' summary.

APITZ, K. [Path. Inst. der Univ. Berlin, Germany] DIE PARAPROTEINOSEN. (ÜBER DIE STÖRUNG DES EIWEISSSTOFFWECHSELS BEI PLASMOCYTOM.) [THE PARAPROTEINOSES (DISTURBANCE OF PROTEIN METABOLISM ASSOCIATED WITH PLASMOCYTOMA).] Virchows Arch. f. path. Anat., 306:631-699. 1940.

This is an exhaustive general discussion of the metabolic disturbances in patients with plasmocytoma. An abnormal protein or paraprotein is produced by the tumor cells where it is demonstrable intracellularly as hyaline protein droplets (Russell's bodies) or in the form of crystalline deposits. These stain red with Congo red. Deposition of this protein in mesenchymal tissues gives rise to nodules of para-amyloid, which in distinction to amyloid, does not have an affinity for the small blood vessels of organs. The protein content of the blood plasma is increased giving rise to albuminuria. A portion of this excreted protein has the characteristics of Bence-Jones albumin. Nephrotic lesions, tubular atrophy, focal interstitial renal sclerosis, and rarely uremia may follow the chemical changes in the kidney.—M. J. E.

BENECKE, E. [Path. Inst. der Univ. Rostock, Germany] ÜBER LEUKÄMISCHE MYELORETIKULOSE MIT ÜBERGANG IN RETOTHELSARKOM. [LEUKEMIC MYELORETICULOSIS WITH TRANSITION TO RETICULUM CELL SARCOMA.] Virchows Arch. f. path. Anat., 306:491-505. 1940.

Two fatal cases of acute myeloid leukemia are described in which the myeloid deposits in the organs were associated with proliferation of reticulum cells. Approximately 25% of the leukemic cells in the circulating blood were monocytes or reticulum cells in various stages of differentiation. The basically neoplastic nature of leukemia is suggested by the sarcomatous character of the reticulum cell proliferation, especially evident in the lymph nodes.—M. J. E.

BICHEL, J. [Aarhus Municipal Hosp., Aarhus, Denmark] ACUTE LEUKEMIA AND "ACHRESTIC" ANEMIA IN A BROTHER AND SISTER. Acta med. Scandinav., 104:578-588. 1940.

Culture *in vitro* of myeloblastic cells of the blood of a patient with fatal acute leukemia revealed the potentiality of the immature cells to develop into mature forms. A short time later the brother of the patient developed achrestic anemia (a blood dyscrasia resembling pernicious and aplastic anemia).—M. J. E.

COSCO, N. P., and H. F. POHLMANN. [Middletown, N. Y.] PRIMARY LYMPHOSARCOMA OF THE TONSIL. New York State J. Med., 41:613. 1941.

Biopsy of the tonsillar tissue of a patient with an atypical unilateral lesion resembling tonsillitis disclosed lymphosarcoma. Radiotherapy was administered and the patient was without evidence of disease 2 years later.—M. J. E.

CRAVER, L. F., R. R. BRAUND, and H. Y. TYLER. [Memorial Hosp., New York, N. Y.] LESIONS OF THE LUNGS IN THE LYMPHOMATOID DISEASES. Am. J. Roentgenol., 45:342-349. 1941.

This report points out the frequency of pulmonary lesions in these diseases and to the variability of their form. Roentgenographic evidence of pulmonary involvement was found in 34% of 282 cases of Hodgkin's disease, 12% of 196 cases of lymphosarcoma, 30% of 13 cases of mycosis fungoides, and 11.8% of 135 cases of leukemia.—E. A. L.

GALL, E. A., H. R. MORRISON, and A. T. SCOTT. [Massachusetts Gen. Hosp., Boston, Mass.] THE FOLLICULAR TYPE OF MALIGNANT LYMPHOMA: A SURVEY OF 63 CASES. Ann. Int. Med., 14:2073-2090. 1941.

Histologic studies have shown a distinct variation in structure in follicular lymphoma, differing from that observed in any other type of malignant lymphoma. In only one case was there evidence of transition into what appeared to be another form of lymphoma. The initial symptoms appear at a much later period of life than with the other malignant lymphomas, the prognosis as to duration of life is considerably longer, and constitutional manifestations and visceral involvement are less frequent. The retroperitoneal lymph nodes are more frequently involved, and chylous ascites is relatively frequent. The lesions are more susceptible to radiation.—H. G. W.

JENSEN, J. P. [Eye Dept. of Odense Amts Og Bys Sygehus] CONJUNCTIVAL LYMPHOMA. Acta Ophth., 18:67-75. 1940.

A case of unusual localization of lymphosarcoma is recorded. Circumscribed subconjunctival tumors were removed by blunt dissection from the fornices of the upper and lower lids of both eyes. Microscopically these consisted of a diffuse proliferation of lymphocytic cells suggestive of lymphosarcoma. The blood picture was normal, and the liver and spleen appeared unchanged. Two weeks later enlargement of the cervical, axillary, inguinal, and femoral lymph nodes was apparent. The histologic structure of an excised node resembled that of the ocular mass. Roentgen therapy eliminated the glandular involvement.—M. J. E.

NELSON, A. A., and H. J. MORRIS. [Division of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D. C.] RETICULUM CELL LYMPHOSARCOMA IN RATS. Arch. Path., 31:578-584. 1941.

Among 323 rats used in chronic experiments were found 35 pulmonary sarcomas of the reticulum cell type, originating in the lymphoid accumulations around the large bronchi. The mediastinal nodes and thymus were never the source of the tumors. Thirty of the 35 rats were over 24 months, and the youngest was 467 days. There were also found in the same series 25 spontaneous extrapulmonary malignant and benign tumors.—H. G. W.

POWELL, W. N. [Scott and White Clinic, Temple, Tex.] THE ACUTE LEUKEMIAS. Tex. State J. Med., 36:486-490. 1940.

A general discussion.—M. J. E.

PUND, E. R., and F. H. STELLING. [Univ. of Georgia Sch. of Med., Augusta, Ga.] LYMPHOSARCOMA. REPORT OF THREE APPARENTLY CURED CASES. Am. J. Surg., 52: 50-54. 1941.

Report of 3 cases with survival periods of 6, 8, and 11 years, without any evident disease in any of the 3 patients. In one, spontaneous regression occurred after surgical relief of obstruction in a lymphosarcoma of the stomach, and the other 2 were cases of early lymph node resection. None of the patients received adequate x-ray therapy.—H. G. W.

TANNHAUSER, S. [Desert Sanatorium, Tucson, Ariz.] LIPOID STORAGE CELLS IN LYMPHOSARCOMA. Arch. Path., 31:378-381. 1941.

A case of lymphosarcoma of the colon is reported which was remarkable from two standpoints: first, the tumor showed the occurrence of great numbers of lipoid storage cells; second, the histologic picture presented features of malignant lymphocytoma and of reticulum cell sarcoma within the same tumor.—H. G. W.

WARREN, S., and J. C. PICENA. [New England Deaconess Hosp., Boston, Mass. and Path. Inst., Rosario, Argentina] RETICULUM CELL SARCOMA OF LYMPH NODES. Am. J. Path., 17:385-391. 1941.

The authors believe that the term reticulum cell sarcoma is at present inexactly used to include almost any lymphoid tumor with proliferation of reticular tissue regardless of degree. This has confused the prognosis and radio sensitivity as reported in the literature. They prefer to follow Oberling's criteria (1932): a tumor composed of syncytical or slightly fenestrated masses of protoplasm containing irregularly distributed oval or indented nuclei with 1 or 2 prominent nucleoli and but little chromatin. Mitoses are present but not in great numbers. Occasional tumor giant cells are found. Reticulum fibers as demonstrated by silver stains may form a dense network of fine scattered fibrils, or be absent in very undifferentiated, syncytial tumors.

Their concept is that the cellular reticulum in a lymph node forms the stroma and is also the stem tissue giving rise to lymphoblasts; therefore a tumor of lymphoid cells contains reticulum cells as part of the tumor itself. From a group of 308 lymphoid tumors only 11 examples of reticulum cell sarcomas were found, a lower incidence than is usually reported. A summary of these 11 cases is appended.—H. B.

WINKELMAN, N. W., and M. T. MOORE. [Univ. of Pennsylvania Grad. Sch. of Med. and Jewish Hosp., Philadelphia, Penn.] LYMPHOGRANULOMATOSIS (HODGKINS' DISEASE) OF THE NERVOUS SYSTEM. Arch. Neurol. & Psychiat., 45:304-318. 1941.

Two cases of involvement of the nervous system by Hodgkin's disease in association with the common generalized type of invasion are recorded. The chief clinical sign in the first patient was a left hemiparesis. There was also evidence of epidural masses involving some spinal roots and the left brachial plexus. Necropsy disclosed invasion of the cerebral dura by Hodgkin's disease, involvement of the right cerebral hemisphere, and multiple areas of softening. The second patient had evidence of a transverse lesion in the region of the sixth dorsal segment and this proved to be a secondary effect of an epidural mass of Hodgkin's disease producing degeneration in the cord.—M. J. E.

Adrenal

ALLEN, P. L. [Weatherford, Tex.] CHROMAFFIN CELL TUMOR ASSOCIATED WITH PAROXYSMAL HYPERTENSION. Tex. State J. Med., 36:540-542. 1940.

Autopsy on a man of 50 years, who died of a hypertensive crisis and gave a history of comparable attacks during previous years, disclosed a pheochromocytoma of the right adrenal gland.—M. J. E.

BISKIND, G. R., M. A. MEYER, and S. A. BEADNER. [Mount Zion Hosp. and Univ. of California Med. Sch., San Francisco, Calif.] ADRENAL MEDULLARY TUMOR. PHAEOCHROMOCYTOMA CURED BY SURGICAL INTERVENTION. CLINICAL MANAGEMENT. ANALYSIS OF ALL REPORTED OPERATED CASES. J. Clin. Endocrinol. 1:113-123. 1941.

A case report of surgical removal of an adrenal medullary tumor is supplemented with details of the pre- and postoperative measures and with a review of the treatment and results in 28 operative cases taken from the literature.—J. B. H.

CARLSON, H. E., and N. F. OCKERBLAD. [Kansas City, Mo.] A CASE OF UNOPERATED HYPERNEPHROMA OF TEN YEARS' DURATION. Am. J. Roentgenol., 45:221-222. 1941.

Report of a case with the original pyelograms and with operative findings 10 years later.—E. A. L.

ELWARD, J. F., and R. L. SPIRE. [Washington, D. C.] BILATERAL HYPERNEPHROMA. Radiology, 35:274-281. 1940.

The pathology of these tumors, and their clinical signs and symptoms are discussed. Five methods of diagnostic roentgenology are used for the localization of renal tumors. They are: roentgenography of the kidneys, pyelography, pneumoperitoneum, perirenal injection of oxygen gas, and examination of the colon and stomach for displacement. Mention is made of the radiosensitivity of cortical renal tumors.

Four cases of bilateral hypernephroma have been reported, 3 with post-mortem examinations. The authors add a 5th case, also with a post-mortem examination. Its unusual features were the lack of subjective symptoms referrable to the urinary tract, the absence of abnormal urinary findings, and the apparent rapid metastases to the mediastinum with localization of symptoms at that point. —E. A. L.

LOEB, M. J. [Bronx Hosp., New York, N. Y.] A CASE OF ADRENAL CORTICAL TUMOR WITHOUT ENDOCRINOLOGICAL SYMPTOMS. J. Urol., 45:785-793. 1941.

Report of an adrenal cortical tumor, with a structure resembling hypernephroma, weighing 1,100 gm., causing no endocrinological symptoms, removed successfully.—H. G. W.

MALLORY, T. B., Editor. [Boston, Mass.] CASE RECORDS OF THE MASSACHUSETTS GENERAL HOSPITAL. CASE 27022. New England J. Med., 224:77-79. 1941.

This is a case report with the incident finding of a small, 1 cm., pheochromocytoma of the adrenal gland. The patient died of a ruptured dissecting aneurysm of the ascending aorta.—A. M.

MARUYAMA, K. [Aus der Inneren Abteilung des Städtischen Krankenhauses Sapporo, Nippon] EIN SEKTIONSFALL DER NEBENNIERENMARKSUBSTANZGESCHWULST BEI EINEM GREISE. [AUTOPSY OF A CASE OF A TUMOR OF THE ADRENAL MEDULLA IN AN OLD MAN.] Gann, 35:27-28. 1941.

A gross and histological description of a highly malignant and widely metastasizing neoplasm of the adrenal medulla in a 66-year-old man is presented. According to the author, a similar case has not been previously reported in the literature.—P. P. C.

MINTZ, N., and S. H. GEIST. [Mount Sinai Hosp., New York, N. Y.] ADRENOCORTICAL SYNDROME. THE ADRENAL CORTEX IN ITS RELATION TO VIRILISM. J. Clin. Endocrinol., 1:316-326. 1941.

Seven cases of virilism are described in moderate detail with the opinion that the adrenal cortex, rather than the pituitary is responsible for the condition. With some omissions in individual cases, the studies include urine analyses, blood counts and chemistry, arterial pressures, basal metabolic rates, glucose tolerance, x-ray of sella turcica and selected parts of the skeleton and abdomen, intravenous pyelogram and perirenal insufflation, endometrial biopsies, visual fields, and microscopical examination of pituitary and adrenal with fuchsinophil stain of adrenal tissue.—J. B. H.

NETTLESHIP, A. [Johns Hopkins Hosp., Baltimore, Md.] ADRENAL MEDULLARY TUMOR. STUDY OF A CHROMAFFIN CELL TUMOR OF THE ADRENAL. J. Clin. Endocrinol., 1:124-125. 1941.

Injection of an extract of one medullary tumor into a female nonpregnant cat indicated that a large amount (67.2 mgm.) of presumed epinephrine was in a 560 gm. adrenal tumor. The tumor was obtained ½ hour after death during an attack of hypertension in a 40-year-old woman.—J. B. H.

TENENBAUM, J. [Israel Zion Hosp., Brooklyn, N. Y.] HYPERNEPHROMA ASSOCIATED WITH HYPERPLASIA AND METASTATIC CARCINOMA OF THE ADRENALS. Am. J. Surg., 52:120-128. 1941.

A rare case is presented of hypernephroma associated with multiple calculous disease, adrenocortical hyperplasia, and metastatic carcinoma of both adrenals, in a woman with marked virilism and facial hypertrichosis.—H. G. W.

TYLEC, L. L. [St. Mary's Hosp., Waterbury, Conn.] HYPERNEPHROMA WITH METASTASES TO THE SCAPULA. Connecticut M. J., 5:432-433. 1941.

A case report.—G. DeB.

WEINBERG, T. [Mt. Sinai Hosp., New York, N. Y.] CONTRALATERAL ADRENAL ATROPHY ASSOCIATED WITH CORTICAL ADRENAL NEOPLASMS. New York State J. Med., 41:884-886. 1941.

Cortical adenomas were excised from 2 patients with Cushing's disease. Both died postoperatively of adrenal insufficiency attributable to atrophy of the contralateral gland. The latter finding was corroborated at necropsy. This patient died of a cortical cell carcinoma which metastasized to the liver and lungs. The cortex of the opposite adrenal was distinctly narrowed.—M. J. E.

Pancreas

BALLINGER, J. [Montefiore Hosp., New York, N. Y.] HYPOGLYCEMIA FROM METASTASIZING INSULAR CARCINOMA OF ABERRANT PANCREATIC TISSUE IN THE LIVER. Arch. Path., 32:277-285. 1941.

An unusual case, described by the title. This is the 8th case of carcinoma of the islands of Langerhans, and the 4th with metastases.—H. G. W.

BROWN, S. J., E. McCARTHY, and A. FINE. [Cincinnati, Ohio] PANCREATIC TUMORS. Radiology, 36:596-603. 1941.

The anatomical position of the pancreas and the superimposition of several organs in this region, each of which may be the site of a neoplasm, make the diagnosis of a pancreatic tumor difficult. Roentgenologic changes in the contour, shape, and position of the stomach and duodenum aid in establishing the diagnosis. These structures usually are displaced forwards and a deformity may be present along the greater curvature of the stomach.—E. A. L.

BURTNESS, H. I., A. E. KOEHLER, and J. H. SAINT. [Sansum Clinic, Santa Barbara, Calif.] HYPERINSULINISM DUE TO ADENOMA OF THE ISLETS OF LANGERHANS. Ann. Int. Med., 14:1915-1932. 1941.

A case report, with symptomatic cure following removal of the tumor.—H. G. W.

FLINN, L. B., G. A. BEATTY, M. GINSBERG, and F. A. HEMSATH. [Delaware Hosp., Wilmington, Del.] CARCINOMA OF THE ISLANDS OF LANGERHANS, WITH HYPOGLYCEMIA AND METASTASES TO THE LIVER. J. A. M. A., 117:283-285. 1941.

Case report.—H. G. W.

FLYNN, J. M. [Rochester, N. Y.] CARCINOMA OF THE HEAD OF THE PANCREAS. Am. J. Roentgenol., 45:380-381. 1941.

Case report.—E. A. L.

FRANCO, S. C. [Long Island Coll. Hosp., Brooklyn, N. Y.] CARCINOMA OF THE HEAD OF THE PANCREAS. Am. J. Digest. Dis., 8:65-69. 1941.

A review of 40 cases, 14 of which were autopsied.—H. G. W.

FRANTZ, V. K. [Presbyterian Hosp., New York, N. Y.] TUMORS OF ISLETS OF LANGERHANS WITH HYPERINSULINISM. New York State J. Med., 41:881-883. 1941.

Operation on 21 patients with hyperinsulinism and resultant hypoglycemia disclosed islet cell tumors in 19. Excellent results were obtained by resection of the growths.—M. J. E.

KAUER, J. T., and F. GLENN. [Cornell Univ., Sch. of Med., New York, N. Y.] CARCINOMA OF THE PANCREAS. Arch. Surg., 42:141-155. 1941.

The authors report an analysis of 32 cases of carcinoma of the pancreas, which was proved either at operation or at autopsy. Contrary to the usual textbook description, the authors found pain to be the most common chief complaint and often the first symptom. In favorable cases, they advise a one-stage radical type of operation with conservation of the external secretion of the pancreas. This consists of a bloc dissection of the head of the pancreas, the adjacent duodenum, and the regional lymph nodes. The transected end of the remaining portion of the pancreas is then transplanted into the posterior wall of the stomach. A cholecystgastrostomy or a cholecystenterostomy establishes a new pathway for the bile, and a gastrojejunostomy is made to reestablish the continuity of the stomach and intestine.—G. DeB.